The absorption of drugs from the site of administration.

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INTRODUCTION

In the last decade, as a result of the discovery and application of new chemotherapeutic agents, there has been a great improvement in the treatment of infections. The two main lines of this development have been with synthetic drugs, particularly derivatives of sulphanilamide, and with substances produced by micro-organisms, particularly penicillin.

Although sulphanilamide was first synthesised in 1908 by Gelmo, it was not until 1935 that Domagk (1935a) published the first report of the effect of azo dyes derived from sulphanilamide on experimentally infected animals; the chemotherapeutic effect was manifested not *in vitro* but only *in vivo*. Tréfouel, Tréfouel, Nitti, and Bovet (1935) were the first to show that sulphanilamide itself has a chemotherapeutic effect, both *in vivo* and *in vitro*. Since then, a large number of compounds related to sulphanilamide have been synthesised, and many of them have been used in the treatment of infections. In 1944, Evans, Fuller, and Walker (1944) described compounds, sulphonyl derivatives of benzamidine, which are particularly effective against the organisms which cause gas gangrene.

The first observation that certain micro-organisms inhibit the growth of others, was made by Pasteur and Joubert in 1877. Although their observation was confirmed by many workers, it was not until 1940 that investigators in Oxford (Chain et al, 1940) were able to purify, and study the chemotherapeutic properties of penicillin, a product of the mould Penicillium notatum, first described by Fleming (1929), and to subject it to successful clinical trials.

In order to obtain the maximum benefit from these new therapeutic weapons, which are characterised by their ability in low concentrations to inhibit the growth of many pathogenic bacteria, and their low toxicity to higher organisms, it is necessary to administer them in such a way that an effective concentration of the drug is reached at the site of infection. Since the action of sulphonamides is bacteriostatic rather than bacteriocidal, it is also necessary that these concentrations should be maintained for considerable periods in the presence of the infecting organisms.

Owing to their low local toxicity it has often been found possible to apply the drugs directly to the site of infection, even apart from the case of surface wounds. Local application is particularly valuable in diseases where the focus of infection

is not reached in effective concentrations when the drugs are given by general administration, and in the case of the sulphonamide drugs it offers the advange that the whole body is not flooded with the drug and general toxic effects are thus avoided.

There are many diseases in which local application has been used. For example, penicillin has been injected intrathecally (Florey and Florey, 1943; Rammelkampf and Keefer, 1943; Evans,1944; Pilcher and Meecham, 1944; Baird, 1945.) and intraventrically (Duthie et al, 1944; McClune and Evans, 1944.) in the treatment of meningitis;sulphonamides and penicillin have been injected into the peritoneal and pleural cavities (Grab, 1943; Jentzer and Calame, 1943; Roberts et al,1945); penicillin has been injected into infected knee joints (Blundell-Jones, 1945) and into the breast in cases of acute mastitis (Weirether et al, 1945): sulphonamides and penicillin have been used extensively by local application in nasal and sinus infections(Fabricant, 1943; Bordley et al,1942; Swanson and Baker,1944); infections of the respiratory tract have also been treated by inhalation of aerosols of pencillin and sulphonamides (Mutch and Hoskins, 1944; Knott and Clark, 1945.). It must be noted however, that

Mc.Adam et al (1945) have reported that adequate concentrations of penicillin could be reached in infected serous cavities after the general administration of massive doses of the drug.

A study of the absorption and distribution of the drugs is essential before rational use can be made of these methods of administration, and it is an important aid in the assessment of their clinical value. In the present study, the drugs have been applied under various conditions to the eye and skin.

The earliest studies in the penetration of drugs through the cornea were made by von Graefe (1854) and Rüte (1864) who found that atropine and eserine penetrated through the cornea into the anterior chamber. When sulphonamides were first used in the treatment of infections of the eye (Guyton, 1940; Mc.Callan, 1940.). they were given orally. It was found that after oral administration, the concentrations of sulphathiazole and sulphadiazine in the ocular tissues were much lower than those of sulphanilamide, while the values for sulphapyridine lie between the two extremes (Bellows and Chinn, 1939, 1941; Chinn and Bellows, 1940, Liebman and Newman, 1941.). Owing to its low solubility, the local application of saturated solutions of drops of sulphanilamide produces only low and ineffective concentrations of the drug in the ocular tissues,

(Luo and P'an, 1940). Saturated solutions of the less soluble drugs, sulphathiazole, sulphapyridine, and sulphadiazine penetrate the cornea even less (Chinn and Bellows 1942; Scheie and Souders, 1941), but better penetration has been reported by the use of a microcrystalline suspension of sulphathiazole (Leopold and Scheie, 1943). High concentrations of the drugs can be reached in the ocular tissues following the application of powdered sulphanilamide or sodium sulphapyridine to the conjunctival sac, but congestion and chemosis are found in the conjunctiva, particularly with the sodium salt, (P'an, 1941a, 1941b). The application of drops of sodium sulphapyridine also causes undesirable reaction (Robson and Scott, 1944), due to the alkalinity of the solution. These results are confirmed by studies in the acid-base tolerance of the cornea, which show that pHs. outside the range 3.5 to 9.5 cause reaction in the cornea (Friedenwald et al, 1944). The solubility, and acid and basic dissociation constants of sulphacetamide are such that its sodium salt is freely soluble at neutral pHs. Robson and Scott (1942, 1943, 1944) have described the beneficial effect of the application of drops of sodium sulphacetamide to the conjunctival sac in experimental infections of the cornea, and Robson and Tebrich (1942) have shown that after a single application of a 30 per cent (W/V) solution of sodium sulphacetamide to the eyes of rabbits, chemotherapeutic levels of the

drug are reached in the cornea, conjunctiva, and rectus muscle.

The local application of solutions of penicillin in the treatment of inflammatory infections of the eye is now generally accepted (Brown, 1946). Struble and Bellows (1944) have shown that only low concentrations of penicillin can be detected in the ocular tissues after the intravenous injection of a massive dose of the drug, and that higher concentrations can be reached by local application.

Experimental and clinical evidence has shown that the topical application of the drugs is particularly effective in the diseases of the anterior segment of the globe. In the treatment of corneal ulcers in which deeper layers of the cornea are involved, or in infections of the posterior tissues and fluids, instillation of drops may not be adequate. Methods of attaining higher concentration in the ocular tissues and deeper penetration are desirable, not only to reach deeper foci of infection, but also to maintain for longer periods, therapeutic concentrations of the drugs.

A large proportion of a solution applied to the eye in drops drains away through the lachrimal duct, and is not maintained in contact with the cornea for very long. Higher concentrations of the drugs are found in the ocular tissues when the solutions are applied continuously to the cornea.

Various forms of corneal baths have been described for this purpose (Robson and Tebrich, 1942; Struble and Bellows, 1944, 1946), and the continuous application of penicillin to the cornea from cotton packs saturated in the drug has also been described (Von Sallman, 1945). A marked increase in the penetration of the drugs into the cornea and aqueous can be achieved by iontophoresis (Boyd, 1942; von Sallman 1945).

Bellows and Gutman (1943) and Leopold and Scheie (1943) have shown that the incorporation of wetting agents into sulphonamide pastes, ointments, and microcrystalline suspensions, applied to the cornea increases the penetration of the drugs into the anterior chamber.

High concentrations of the drugs are found in the aqueous and ocular tissues after subconjunctival injection (Von Sallman, 1945; Leopold and Scheie 1943; and Rycroft, 1945b). Von. Sallman (1945) has shown that iontophoresis and cotton pack application are more effective than subconjunctival injection in producing high concentrations and deeper penetration of penicillin into the ocular tissues. Leopold and La Motte (1945) have shown that where the cornea is abraded, effective concentrations of penicillin are reached in the aqueous after the instillation of drops, without resort to the use of iontophoresis or subconjunctival injection.

By these methods only very slight or indetectable concentrations of the drugs are found in the vitreous;

small amounts of penicillin are found in the vitreous after subconjunctival injection (Leopold 1945a; Rycroft, 1945). Effective concentrations of the drug are found in the vitreous after the injection of penicillin directly into the anterior chamber (Leopold, 1945a), and high concentrations are maintained in the vitreous after the intravitreous injection of sodium sulphacetamide and penicillin (von Sallman et al: 1944; Rycroft, 1945a; Mann, 1946).

The present work is mainly concerned with the action of wetting agents in increasing the penetration of drugs into the eye, and, with the distribution of drugs in the ocular tissues after intravitreous injection.

One of the first conditions to be successfully treated with a sulphonamide was a skin infection (Schreus, 1935). In dermatology the sulphonamides were first used orally, and it was only later that their external use was suggested. In a review Cole (1943) cites many reports describing the successful use of sulphonamides by local application in skin infections. At that time evidence was appearing which revealed that sensitisation to the general administration of sulphonamides is produced by the local application of the drugs to the skin. Indeed Benedick (1946) has recently stated that "the topical use of sulphonamide is considered as strictly contraindicated for the treatment of superficial infection of the skin".

Most of the studies of the absorption of sulphonamides after local application to the skin have been made by estimations of the concentrations of the drug reached in the blood. These results indicate that after local application to intact skin, only small or even indetectable amounts of the drug are found in the blood. (Pillsbury et al 1941; Robinson and Robinson, 1941). Kalz and Prinz (1942) found that there was a considerable increase in absorption if the skin was deprived of the stratum corneum and that the highest levels were obtained with broken skin.

Strakosch and Clark (1942, 1943a, 1943b) have estimated the concentrations of sulphonamides in the skin after the local application of ointments and wet packs. They found that the type of emulsion in the ointment base did not effect the concentration of the drug in the skin. The sulphonamides penetrated better into the skin from wet packs than from ointments, particularly when the skin was damaged. The drugs are retained by the intact skin for as long as six days after the local application was stopped.

Mc.Kee et al (1943a,1943b) have used histochemical methods to study the penetration of locally applied sulphonamides into the skin. They have described bases containing detergents, xylene, antipyrene, and propylene glycol, from which the drugs penetrate deeper into the skin.

In the present work a study has been made on the

effect of various factors on the concentrations of sulphacetamide found in the skin and superficial tissues after the local application of sodium sulphacetamide to the skin.

METHODS.

1. Biological Methods.

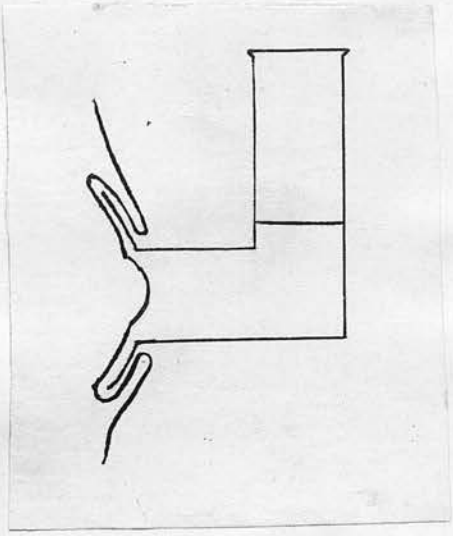
In all experiments mature animals (rabbits, guinea pigs and rats) of both sexes and various breeds were used.

(a) Application of drops to the eye. 0.1 ml. of the drug solution is instilled into the conjunctival sac of a rabbit's eye, and the lids are then gently closed and opened for twenty seconds. After a lapse of five minutes, 0.1 ml. of another solution is applied under the same conditions to the other eye of the same rabbit. Fifteen minutes, one hour, or three hours after the instillation, the rabbit is killed and the eyes excised. After enucleation, the aqueous is aspirated, and the conjunctiva, cornea, iris and sclera, are removed. The tissues are washed rapidly in saline and dried on blotting paper, and are then weighed for analysis.

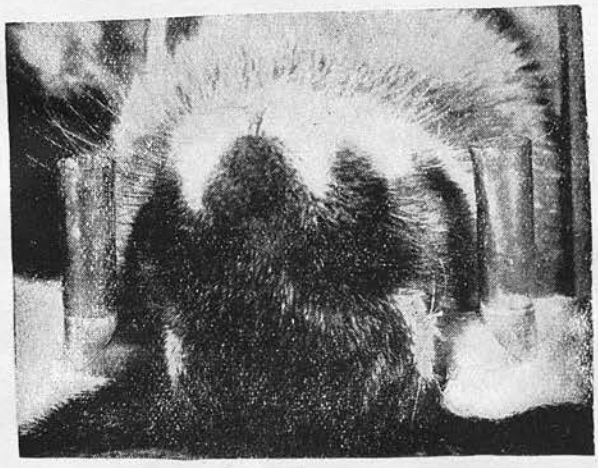
(b) Continuous application of drugs to the eye in situ.* In these experiments right-angled celluloid funnels with a diameter slightly greater than that of the cornea of a rabbit and with a curved flange at one end, are used. The curvature of the flange is such that it fits comfortably into the conjunctival sac of a rabbit, where the funnels are placed under ether anaesthesia (Figures 1a and b). They are kept in position by

* The method described by Robson and Tebrich (1942)

Figure 1



(a)



(b)

Showing the method for the continuous application of drugs to the eye, in situ.

means of purse string sutures through the lids. The flanges lie against the conjunctiva overlying the sclera, and no part of the funnel is in contact with the cornea. The amount of solution put into the funnel (3 ml.) is such that the cornea is not subject to any appreciable hydrostatic pressure. After filling the funnel, all air bubbles are carefully removed. The drug solution is maintained in direct contact with the cornea and fills the conjunctival sac. The drugs are applied for six or fifteen minutes, at the end of which, the funnels are removed and the eyes excised and dissected as before for analysis.

This method is also applicable to excised eyes. The funnel is sewn in place in a dead animal, (immediately after killing), and incisions are made through the lids so that the eye together with the lids is excised with the conjunctival sac intact, and the funnel in position. The funnel is clamped vertically and the eye is supported on a pad of cotton wool soaked in saline; the drug is then applied as described above.

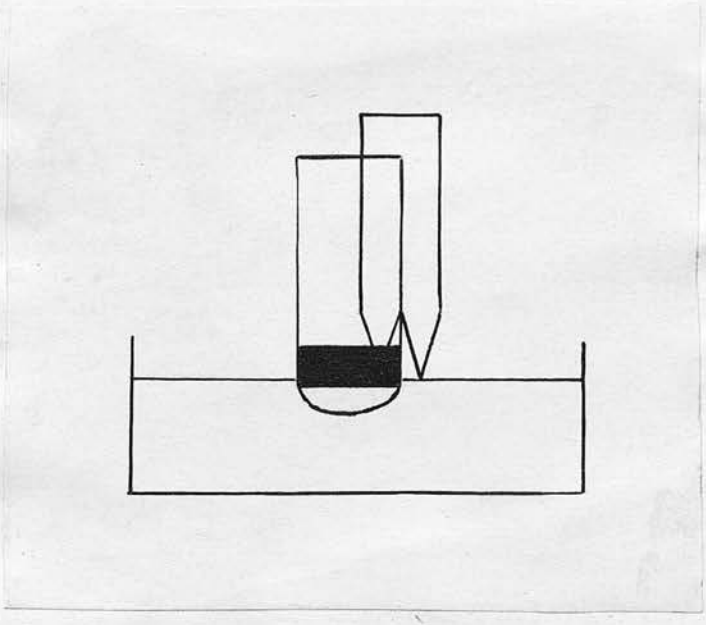
(c) Continuous application of drugs to isolated ocular tissues in vitro. Throughout the experiments in vitro, the excised eye, or parts of the eye, are kept moist by bathing as much as possible in a solution similar to the anterior chamber fluid (Duke-Elder, 1927), and containing the

following substances in the following concentrations.

Sodium chloride	0.71 gm. per 100 ml.
Potassium chloride	0.038 gm. per 100 ml.
Calcium chloride	0.017 gm. per 100 ml.
Glucose	0.1 gm. per 100 ml.

In these experiments, the eye is excised from a newly killed rabbit and is cleared of conjunctiva and rectus muscle. It is then opened at the posterior pole so that the cornea and sclera together form a sac from which the lens and iris are removed, and then completely cleared of choroid and retina by wiping away the chorioretinal layer with cotton wool. The sac is drawn over the end of a small glass tube (1.3 am x 3.5 cm) which has a small constriction at that end (see Figure 2). The diameter of the tube is slightly less than the diameter of a rabbit's cornea, so that the cornea, excluding the limbus, forms a membrane over the mouth of the tube, The membrane is secured in position by a loop of silk thread tied tightly round the tube at the constriction, which prevents it from slipping, and is firmly bound with elastic insulating rubber, so that only the tissue over the end of the tube is exposed. 0.8 ml. of the anterior chamber solution is placed inside the tube which is then lowered into a solution of the drug. In order to prevent a flow of the drug into the tube, or of solution out of the tube, caused by hydrostatic pressure, the solution levels inside and outside the tube have to be adjusted to the

Figure 2



Showing the method for the continuous application of drugs to isolated ocular tissues.

same height. This is done by means of a small perspex slip, cut so that it has two equal legs and a slit which allows it to move freely up and down the side of the tube. The level of the solution inside the tube is the same as that outside when both legs are just touching the solution. Five and fifteen minutes after contact is first made between the tissue and the drug solution, the contents of the tube are stirred by a stream of air from a syringe and 0.1 ml. of the solution is withdrawn for analysis. The tissue in contact with the drug solution is also removed for analysis after fifteen minutes. This method is used for studying the penetration of sodium sulphacetamide through the normal corneae, denuded corneae, corneae from eyes treated with chemical warfare agents, denuded corneae from eyes treated with chemical warfare agents, and normal sclera, all from the eyes of rabbits. The method is similar to that used by Yonge (1936) for studying the penetration of ions through chitin membranes.

(d) Subconjunctival Injection. To a rabbit under deep ether anaesthesia, 0.6 ml. of a drug solution is injected under the ocular conjunctiva, in three blebs of approximately 0.2 ml.. A No. 20 hypodermic needle is used, and sterile precautions are observed. After one, six or twelve hours the rabbit is killed and ocular tissues and fluids are removed for analysis. Immediately after removal,

the tissues are washed rapidly in saline and dried on blotting paper before weighing. Where the drug used is penicillin, in the dissection and aspiration of the ocular tissues and fluids, sterile instruments are used and the tissues are washed in sterile saline and dried on sterile blotting paper and weighed in a sterile vial. Before aspirating the vitreous, the conjunctiva and sclera at the point at which the needle is to be inserted is first sterilised by application of a cautery.

(e) Intravitreous injection. For intravitreous injection a No. 20 hypedermic needle with a shortened stem (7-8 mm; the length of the stem must be less than the diameter of the globe) is used. The syringe is clipped to a special holder so that the plunger is in secure contact with the piston of a micrometer screw gauge. Rotation of the screw forces out the contents of the syringe. Thus small, accurately measured amounts can be injected.

The injection is carried out with sterile precautions, on a rabbit under deep ether anaethesia. The superior rectus muscle is firmly held in fixatation forceps, and the adjacent sclera under the ocular conjunctiva is punctured. 0.1 ml. of the drug solution is then delivered into the vitreous. Great care is taken to prevent the needle from puncturing the lens. In cases where

conjunctival tissue is required for analysis, the needle is wiped free of the drug with sterile blotting paper and the conjunctival sac is thoroughly washed out with sterile saline. Thus concentrations of the drugs found in the conjunctiva cannot arise from drug solution adhering to the outside of the needle. Sterile precautions are observed, as already described, in the dissection of the tissues after intravitreous injection of penicillin.

(f) Methods used to damage eyes.

(i) Application of chemical warfare agents. The violent toxic affects resulting from the contamination of eyes with vesicants used in chemical warfare are well known. Agents used for this purpose were dichlorethyl sulphide (mustard gas; it will be referred to as H), and N methyl di (2 chloroethyl) amine (which will be referred to as NH). H is applied in a 1 per cent solution in liquid paraffin B.P., and NH is applied as a 1 per cent solution of its hydrochloride in saline. 0.025 ml. of the solution of the vesicant is placed on the upper part of the cornea of a rabbit, and is allowed to flow over the whole surface of the cornea.

(ii) Denudation of the cornea. The epithelium of the cornea of a rabbit under deep ether anaesthesia is completely removed by scraping

with a fine knife; the eye is held steady by fixation forceps applied outside the limbus. Care is taken not to damage the substantia propria. Sterile precautions are observed.

(iii) Experimental corneal infections. The method used is that described by Robson and Scott. (1943) Immediately before the experiment a 24 hour culture of the organism is diluted to contain 1,500 organisms per ml.. The rabbit is deeply anaesthetised with ether and the injection is performed with a No. 20 hypodermic needle with a small bevel at the point. The culture is injected to form a small bleb under the corneal epithelium, the eye being fixed in position by fixation forceps applied outside the limbus. A sterile technique is observed. Robson and Scott used a culture of Staphl. aureus which was obtained from infected human eyes, in the present experiments the Oxford H strain of Staphl. aureus is used.

(iv) Experimental infections of the vitreous. Immediately before the experiment, a 24 hour culture of the organism is diluted 1:1,000,000. 0.02 ml. of the suspension is injected by the method described, into the vitreous of a rabbit. The organism used is Haemolytic streptococcus (strain lc. - as described by Robson and Scott, 1944).

(g) Application of drugs to the skin in vitro. The method is essentially the same as that

described for the penetration of drugs through ocular tissues (p.13). A piece of skin from which the hair had been removed, about 3 $cm.^2$, is taken from the abdomen of a newly killed animal. The fascia is dissected off, and the skin, with the external surface to the outside is drawn over the end of the small glass tube and attached as already described in the case of the ocular tissues. Throughout these experiments, the internal surface of the skin is moistened as much as possible with mammalian Locke's solution. I ml. of Locke's solution is introduced into the tube which is then lowered into a solution of the drug, and the levels of the solutions inside and outside the tube are adjusted as already described. After 15 minutes the tube is raised, the contents of the tube are mixed, and 0.5 ml of the solution is removed for analysis. The skin which had been in contact with the solution is also removed, washed rapidly in saline and dried on blotting paper before weighing for analysis.

Experiments of this type were used to determine which method of removing hair from the skin least affects the penetration of drugs through the skin. The methods used to remove the hair were, by shaving, by plucking, by trimming with scissors, and by the use of a depilating paste of the following composition:-

Barium sulphate	250 gm.
Castille soap powder	50 gm.
Talcum powder	350 gm.
Flour	350 gm.

Make a thick paste with water.

On the day before the skin is required, the hair is trimmed with scissors, and the skin is treated with the paste for three minutes in the case of rabbits and two minutes to guinea pigs. The paste is then washed off thoroughly with warm water. It is very effective. In all cases and with all methods of removing the hair, the hair is removed one day before the skin is required.

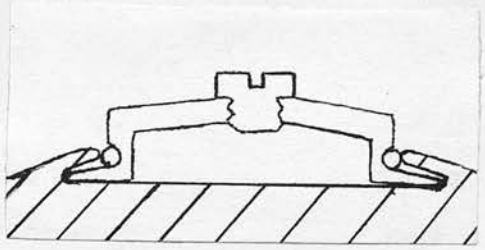
The in vitro method is also used to compare the penetration of drugs through the skin of various species - rabbit, rat, guinea pig and human skin. The human skin was obtained from breasts which had been amputated in surgical operations.

(h) Application of drugs to the skin in vivo for long periods.* In these experiments the drug solutions are applied to the skins of rabbits and guinea pigs from a perspex cap. The cap has the shape of a 'boater' straw hat; the 'crown' diameter being 2.5 cm., and the 'brim' diameter 3.5 cm. . At the 'hat-band' there is a slight groove into which fits a loop of steel spring which is slipped over the 'crown'. The skin of an anaesthesised animal is sterilised with alcohol and 40 per cent iodine in alcohol. The cap is attached to the skin by tying silk sutures through the skin to the turns of the steel spring. The position of the cap is first fixed by sutures at twelve, six,

* Method devised by Dr. J.M. Robson.

three, and nine o'clock and the remainder are attached sector by sector. The solution of the drug is introduced through a screw hole at the centre of the 'crown'. Folding of the skin between sutures results in leakages and is avoided as much as possible. Friar's balsam is applied to the silk sutures to prevent them from snapping during the experiment. Figure 3a is a cross-section of a cap in position and shows how the skin folds over the extremity of the 'brim' making a water-tight compartment. Figure 3b shows the cap in position in a rabbit. When the period of application of the drug has elapsed, the contents of the cap are removed by suction and the skin with the cap in position is washed once with saline, which is also withdrawn by suction. The cap is then taken off by cutting the sutures, and after drying with blotting paper about 100 mg. of skin with its superficial fascia is removed for analysis from the area under the cap, and immediately adjacent to it. The fascia and skin are separated, washed rapidly in saline, and dried on blotting paper before weighing. In one day experiments on rabbits, 0.01 ml. samples of the drug solution were removed from the cap for analysis, zero, one, two, three, four, five, and twenty four hours after the application of the drug; in three day experiments on guinea pigs, samples were

Figure 3



(a)

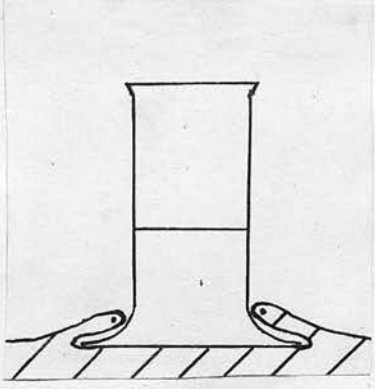
(b)

Showing the method for the continuous application of drug solutions to the skin, for long periods *in vivo*.

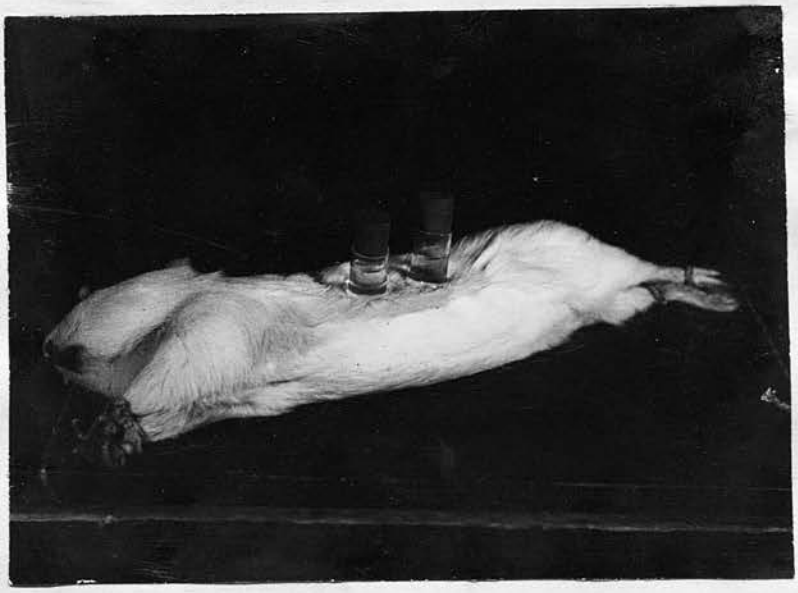
taken zero, six, twenty four, thirty, forty eight, fifty four, and seventy two hours after the application of the drug. The perspex caps were also used to produce experimental infections of the skin. After sterilisation with alcohol and iodine, ten parallel cuts are made through half the depth of the skin. The scarified area is then covered with the cap. When it has been sewn in position, 0.5 ml. of a suspension of the infecting organism is placed on the incisions, and the cap is filled with sterile saline. The silk thread and the needles used are sterilised by heat, and the caps, which warp in boiling water, in a solution of phenyl mercuric nitrate which is washed off with sterile water before use. A suspension of a diphtheroid of the Hofman group is effective in producing a clinical infection and pus formation in one day, when applied in this way.

(i) Application of drugs to the skin for short periods, in vivo. These experiments were performed on guinea pigs. The drug is applied in a small glass tube 1.7 cm. in diameter, with a 3 mm. flange at one end. A circular purse-string suture of stout silk with diameter slightly greater than the flange diameter is sewn through the skin on the side of a guinea pig, under ether anaesthesia. The tube is placed inside the suture with the flange on the skin, and on tying the suture the tube is held

Figure 4



(a)



(b)

Showing the method of application of drugs to the skin for short periods, *in vivo*.

firmly in position. Figure 4a shows a cross section of a tube in position, and Figure 4b is a photograph of two tubes in position on the side of a guinea pig. The drug solution is placed in the tube, and air bubbles which form at the surface of the skin are dispersed. After fifteen minutes the drug solution is rapidly withdrawn by suction, and the skin is washed twice by refilling the tube with saline. When the second wash of saline has been removed by suction, the suture is cut and the tube is taken off. The skin which has been in direct contact with the drug is dried with blotting paper and is dissected off with the superficial fascia. The fascia and skin are parted and weighed separately for analysis. Skin and fascia immediately adjacent to the tube are sometimes taken for analysis.

These experiments are carried out on normal skin, skin which had been treated for 24 hours with saline, infected skin, scarified skin, and grazed skin. The skin is treated with saline and infected as already described (see p. 21). The skin is scarified with ten parallel cuts through half its depth immediately before the attachment of the tube. The skin is grazed by stroking a fold of the skin held between the thumb and forefinger with coarse sandpaper - ten strokes remove the stratum corncum without rupture of any blood vessels; twenty strokes produce more severe damage with rupture of blood vessels.

(2) Physical measurements.

(a) Measurement of diffusion constant (Fick's constant). The diffusion constant of sulphonamide drugs diffusing through agar and gelatin gels have been measured by Hawking (1941). The method used by Hawking is adapted more to the measurement of the length of gel through which the drug diffuses rather than an accurate determination of the diffusion constant. The object in the experiments to be described is to determine whether dodecyl sodium sulphate affects the rate of diffusion of sodium sulphacetamide through gels.

The diffusion constant is defined as the weight of material passing in unit time across a plane, 1 sq. cm. in area, when the concentration gradient across the plane is unity. Hence the quantity 'dw' diffusing across a plane of area 'a' in time 'dt' and where the concentration gradient is '-dc/dx', is given by the expression

$$dw = -D.dc/dx.dt$$

from which the diffusion constant D may be calculated (Glasstone, 1940).

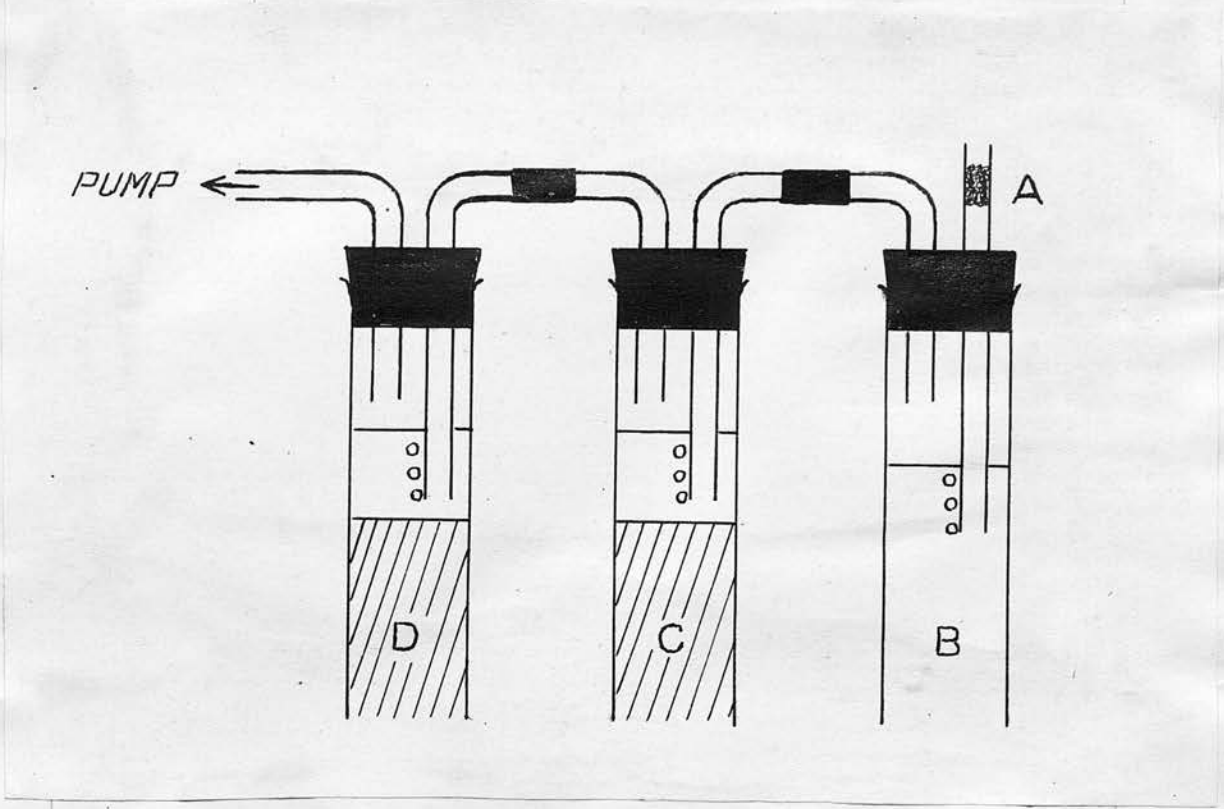
Gelatin gels are prepared containing 2 gm., 5 gm., 10 gm., or 20 gm. of Cox's gelatin ~~per 100ml~~., and 3 gm. of sodium sulphacetamide, and in some cases 0.1 gm. of dodecyl sodium sulphate, in 100 mil. distilled water.

The gels are allowed to set for twenty-four hours.

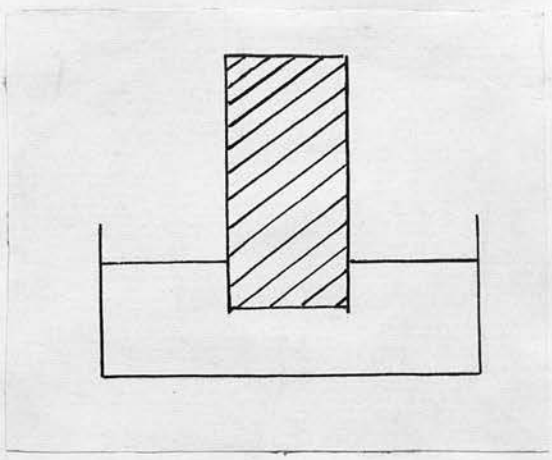
in large test tubes. (3.5 cm x 20 cm). When the gel has set, 10 ml. of 0.9 per cent sodium chloride is placed on the surface of the gel where it is agitated by a stream of air. Figure 5a shows the form of the apparatus. The air is drawn by a suction pump and first passes through cotton wool (a) and then through a tube containing distilled water (b). The tube c contains a gel incorporating sodium sulphacetamide, and tube d, contains a gel of the same concentration incorporating both sodium sulphacetamide and dodecyl sodium sulphate. Ninety minutes after the saline is placed on the surface of the gel, 5 ml. of it is removed for analysis. The area of cross-section of the tube at the saline-gel surface is obtained by measuring the height of the 10 ml. of saline in the tube. Great care is taken to prevent solution from one tube being drawn over into its neighbour; when the concentration of gelatin is greater than 20 gm. per 100 ml. a foam is produced in the saline which is drawn over by the air, making the experiment valueless.

In tubes with diameter less than those used, the meniscus on the gel surface may cause an appreciable difference between the area of cross-section of the tube and the area of the gel surface.

Figure 5



(a)



(b)

Showing the methods used for measuring the diffusion of drugs out of, and into gelatin gels.

In the calculation the assumption is made that the gel extends to an infinite distance beyond the surface. For the period of time chosen, the depth of the gel should be at least 10 cm. in order to make negligible errors due to this assumption.

The diffusion of sodium sulphacetamide was studied under conditions more akin to those under which the drug diffuses into tissues, to which it is applied locally. A small cylindrical sample tube (2.0 cm. x 7.5 cm.) is filled with a 5 gm. per 100 ml. solution of gelatin in water, which is allowed to set for 24 hours. The tube is then inverted with the open end immersed 2 cm. below the surface of a 10% (W/V) solution of sodium sulphacetamide in water, or in 0.1 (W/V) dodecyl sodium sulphate in water. One hour after immersion the tube is removed and is heated to liquefy the gel. The volume is made up to 100 ml. with 15 per cent trichloroacetic acid and after filtering the precipated protein, the sulphonamide content of the filtrate is estimated.

(b) Measurement of interfacial tensions.

The method is the well known method which depends on the measurement of drop weights (or drop volumes). Since the surface tension of the solution is examined, (aqueous solutions of wetting agents), depend on the age of the

surface (Gaddum, 1931) there is no attempt at absolute measurement of the surface tension. The drop size of a solution delivered from a nozzle is compared with the drop size of another solution from the same nozzle when the drops of both solutions are formed in the same time.

Drops formed in air:- Ten drops of the solution are delivered into a weighing bottle from a 30 ml. burette containing 10 ml. of the solution and are weighed. The time of delivery is kept constant at approximately 200 seconds for ten drops.

Drops formed in liquid paraffin:- Ten drops of the solution are delivered from a 5 ml. micro burette with the tip immersed 1 cm. below the surface of liquid paraffin B.P. in a large crystallising dish. The total volume of solution delivered is too small to affect considerably the level of paraffin in the dish, and thus the drops are formed under the same pressure of paraffin. The time for delivery of ten drops is kept at approximately 200 seconds and the volume of ten drops is measured (the significance of drop weight measurement in this case would be lessened by the indeterminate amount of paraffin which adheres to the burette).

In both cases, the height of the solution in the burette is adjusted to the same mark before the delivery of the drops is started, so that the drops

are formed under the same pressure from the solution in the burette.

(3) Analytical Methods.

(a) The estimation of sulphanilamde derivatives.

There are two general methods for the colorimetric estimation of sulphemamides, or aromatic amines generally. One depends on the condensation of the amine with dimethylaminebenzaldehyde (Ehrlich's aldehyde) to form a yellow compound (Morris, 1940), and the other on the production of an azo dye. In the latter method the amine is diazotised and then coupled with a suitable compound to give a brightly coloured dye. Compounds used as coupling agents include dimethyl-α-naphthylamine (Marshall, et al, 1937), chromotropic acid (Scudi, 1937) and β naphthol (Fuller, 1937).

The method adopted is the diazotisation method of Bratton and Marshall (1939) in which the coupling component is N(1 naphthyl) ethylene diamine dihydrochloride. The colour produced is bright red which is more suitable than yellow for visual colorimetric comparison, and the coupling can take place over a comparatively wide range of hydrogen ion concentrations.

Extraction of sulphonamides from tissues.

Marshall, Emerson, and Cutting (1937) used acetone to extract sulphonamides from tissues; Bellows and Chinn (1939) ground the tissues with sand in 15 per cent trichloroacetic acid. The latter

method is the simpler and was adopted for tissues other than skin. Owing to the resistance of skin to grinding, the method of Bellows and Chinn is very labourious and therefore not entirely satisfactory. Strakosch and Clark (1943a) have extracted sulphonamides from the skin by heating the tissue in 2N hydrochloric acid at 100° C for thirty minutes, followed by vigourous shaking until the skin is completely macerated.

Reagents

Trichloroacetic Acid A.R.	15 gm. in 100 ml distilled water
Silver sand	
Hydrochloric acid A.R.	2 Normal solution in dist. water.
Sodium nitrite A.R.	0.1 gm. in 100 ml. in dist. water.
Ammonium sulphamate B.D.H.	0.5 gm in 100 ml. dist. water
N(1 naphthyl) ethylene diamine dihydrochloride	0.1 gm. in 100 ml. dist. water
Saponin	0.5 gm. in 1000 ml. dist. water
Stock solution of sulphonamide	200 mg. per 1000 ml. dist. water.

Standard solutions of sulphonamide are made in concentrations of 0.2, 0.5, and 1.0 mg. per 100 ml. To prepare these 5, 2.5 and 1.0 ml. of the stock solution plus 18 ml. of the 15 per cent trichloroacetic acid are diluted to 100 ml. with distilled water.

The weighed tissues are thoroughly ground in about 1 gm. of clean silver sand and 4 ml. of 15 per cent tricloroacetic acid in 2 oz. glass mortars. The resulting suspension is then washed into 25 ml. measuring cylinders and allowed to stand for at least 45 minutes. The total volume

is made up to 25 ml. with distilled water, and after shaking, the suspension is filtered through a No. 2 Whatman filter paper. When the tissue is skin the following technique is used:- After weighing, the skin (about 100 mg.) is placed with 2 ml. of 2N Hydrochloride acid, in a test-tube graduated at 20 ml., and heated for 20 minutes in a boiling water bath. If heating is continued for more than 20 min., the resulting extract is brown in colour, which interferes with the colorimetric estimation. After heating, the test-tube is shaken vigourously until the skin is completely macerated, and 4 ml. of 15 per cent trichloroacetic acid added. After standing for 30 minutes the volume is made up to 20 ml., and the protein precipitated by the trichloroacetic acid is removed by filtering under gravity through a Whatman number 5 filter paper.

To 10 ml. portions of the filtrate (from skin and other tissues) pipetted into 50 ml. conical flasks, 1 ml. of 0.1 per cent sodium nitrite is added. After three minutes during which the flasks are shaken vigourously, 1 ml. of 0.5 per cent ammonium sulphamate is added to destroy the excess nitrite. The flasks are again shaken vigourously and after two minutes, 1 ml. of 0.1 per cent N (1 naphthyl) ethylenediamine dihydrochloride is added. The red colour develops rapidly and the

flasks are shaken once more, and after a pause of two minutes the estimations are made. If the ammonium sulphamate is not added the excess nitrite reacts with the coupling component to form a brown water-insoluble compound. Readings are taken on a Klett B 10 visual colorimeter by comparison with the colours produced in the standard solutions. For concentrations, in the final extract, of less than 0.1 mg. per 100 ml. a Spekker photoelectric absorptiometer is used with the No. 6 filter of the H. 445 set with maximum transmission at 0.54 μ .

Procedure for blood and urine.

In all cases in which blood or urine are taken for analysis the total sulphonamide is estimated (i.e. free plus acetylated sulphonamide). The acetylated compound is first hydrolysed by heating at 100° C with hydrochloric acid; it has been shown that the hydrolysis is not complete unless a reflux condenser is used. (Druey and Osterheld, 1943).

To 2 ml. of oxalated blood, 30 ml. of 0.05 gm. per cent saponin is added and followed two or three minutes later by 8 ml. of 15 per cent trichloroacetic acid. The precipate is filtered off and the total sulphonamide is determined as follows:- 20 ml. of the filtrate is treated with 1 ml. of 4 N hydrochloric acid and heated in a test-tube,

calibrated at 20 ml., under a reflux air condenser on a boiling water bath, for one hour. After cooling, the volume is adjusted to 20 ml. (there is slight loss of water vapour through the condenser) and the drug is estimated in a 10 ml. sample as described above.

Urine is diluted with 15 per cent trichloroacetic acid and distilled water 20-100 times, depending on the concentration of the drug anticipated. The final concentration of trichloroacetic acid is approximately 3 per cent (i.e. the same concentration as in the standards). The sulphonamide in 20 ml. of the diluted urine is hydrolysed and estimated as in the case of blood filtrates. In cases where the concentration of sulphonamide in the filtrate is greater than 2 mg. per 100 ml., the estimation is repeated with a tenfold dilution of the filtrate, in such a way that the concentration of trichloroacetic acid in the final solution is again about 3 per cent.

(b) Estimation of aromatic amidines.

The method employed for the estimation of aromatic amidines is that described by Fuller (1945). By heating a solution of an aromatic amidine with a small amount of glyoxal in the presence of a borate buffer at ph.9., as the result of a complex reaction, a colour is produced, which is suitable for the estimation of amidines in concentrations

as low as 1 part in 100,000. The maximum colour is obtained with about two molecules of glyoxal to one molecule of amidine and excess glyoxal inhibits the reaction. Estimations were made only on extracts from rabbit corneae and conjunctivae. According to Fuller (1945) aromatic amidines are precipitated by the usual protein precipitants, such as trichloroacetic acid and toluenesulphonic acid, and are also absorbed on filter paper. To extract aromatic amidines from tissues, a method similar to the method for extracting sulphonamides from skin is used.

Reagents

Hydrochloric acid A.R.	2 Normal solution in dist. water.
Sodium hydroxide A.R.	2 Normal solution in dist. water.
Borate buffer ph 9	4 gm. of boric acid A.R., are heated in dist. water to dissolve and neutralised to ph 9., and the volume made up to 100 ml.
Glyoxal sodium bisulphite	0.1 gm. in 100 ml. dist. water.

Procedure

After weighing, the tissue is heated in a graduated 10 ml. centifuge tube with 2 ml. 2 N hydrochloric acid in a boiling water bath for 15 minutes. The tube is then shaken vigourously until the tissue is completely macerated, the hydrochloric acid is neutralised with 2 ml. 2N sodium hydroxide and 2 ml. of the borate buffer is

added. The volume is then made up to 10 ml. with distilled water and the tissue fragments are sedimented by centrifuging. To 5 ml. of the clear solution, 0.5 ml. of 0.1 per cent solution of glyoxal sodium bisulphite is added, and after heating for ten minutes in a boiling water bath, the colour develops and readings are taken on a Spekker photo-electric absorptiometer, using the No. 5 filter of the H. 455 set.

(c) Estimation of Penicillin.

The estimations were carried out by Dr. J.P. Duguid by the method described by Mc.Adam, Duguid, Challinor, and Mc.Call (1945).

Extraction of penicillin from tissues. Struble (1944) and Bellows describe the extraction of penicillin from tissues by grinding the tissue with sand in distilled water. In order that the extract fluid should have a sufficiency of nutrient substances so that inhibition of growth of the test organism is not due to "natural stasis", the penicillin was extracted by grinding in horse broth serum.

After weighing in a sterile vial, the tissue is transferred to a sterile agate mortar, where it is ground in sterile silver sand and 0.6 ml. of horse broth serum, with a sterile pestle. The grinding is carried out in a sterile cupboard. The macerated tissue is transferred by a Pasteur pipette to a sterile tube and the mortar and pestle are

washed in horse broth serum which is also transferred to the tube. The volume of horse broth serum used for washing is such that the total dilution of the tissue is 1 in 10. The macerated tissue is centrifuged and the assay is made on the clear supernatant fluid.

Note on Drug Solutions. Solutions of the drugs were made in sterile saline, in the case of penicillin, and in distilled water, with sodium sulphacetamide and V.187 hydrochloride. In order to bring the ph. of the drug solutions to a neutral value, hydrochloric acid is added to sodium sulphacetamide and sodium hydroxide is added to V.187 hydrochloride.

RESULTS.

1. Experiments on the methods of estimation.

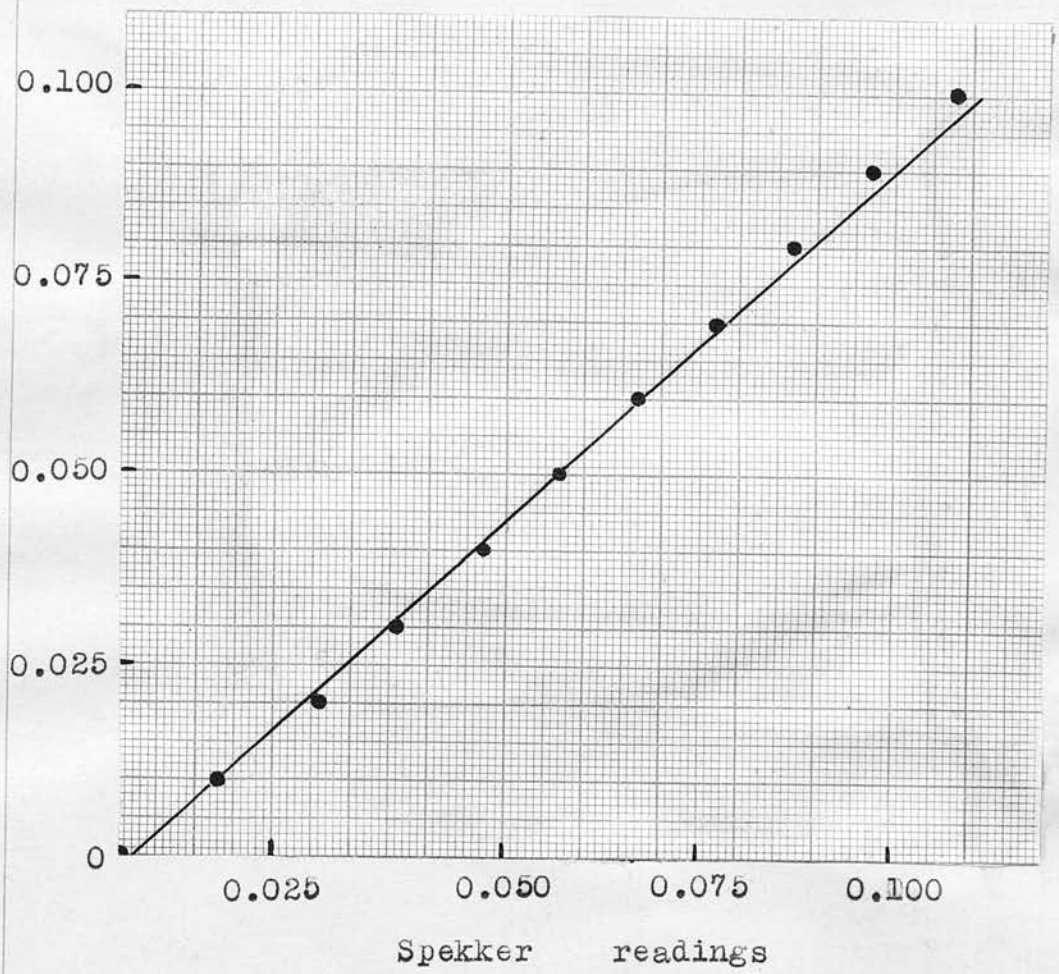
(a) Sulphonamides. Figure 6 shows the Spekker photoelectric absorptiometer calibration curve for sulphacetamide estimated by the method of Bratton and Marshall. The maximum deviation from the straight line is about 3% which may be regarded as the experimental error in the estimation.

Time between grinding and filtration.	Mg. Sodium Sulphacetamide Added.	Mg. Sulphacetamide recovered.	Mg. Sodium Sulphacetamide recovered.	Percentage Recovery.
0	2.5	1.89	2.07	82.8
15 min	1.5	1.18	1.31	87.3
30 "	1.0	0.835	0.914	91.4
45 "	0.5	0.448	0.490	98.0
60 "	1.0	0.903	0.966	96.6
90 "	0.6	0.547	0.599	99.5
150 "	3.0	2.82	3.09	103.0

Table 1 Recovery of added amounts of sodium sulphacetamide to minced mouse tissue, by grinding with 15 per cent trichloroacctic acid.

Table 1 shows the recoveries of sulphacetamide from minced mouse tissue to which known amounts of the drug had been added, and the effect on the recovery of the time between the grinding of the tissues and filtration. It illustrates clearly that the macerated

Figure 6



Spekker calibration curve for the estimation of sulphacetamide.

tissues must be left for at least 45 minutes before filtering, in order to obtain complete extraction.

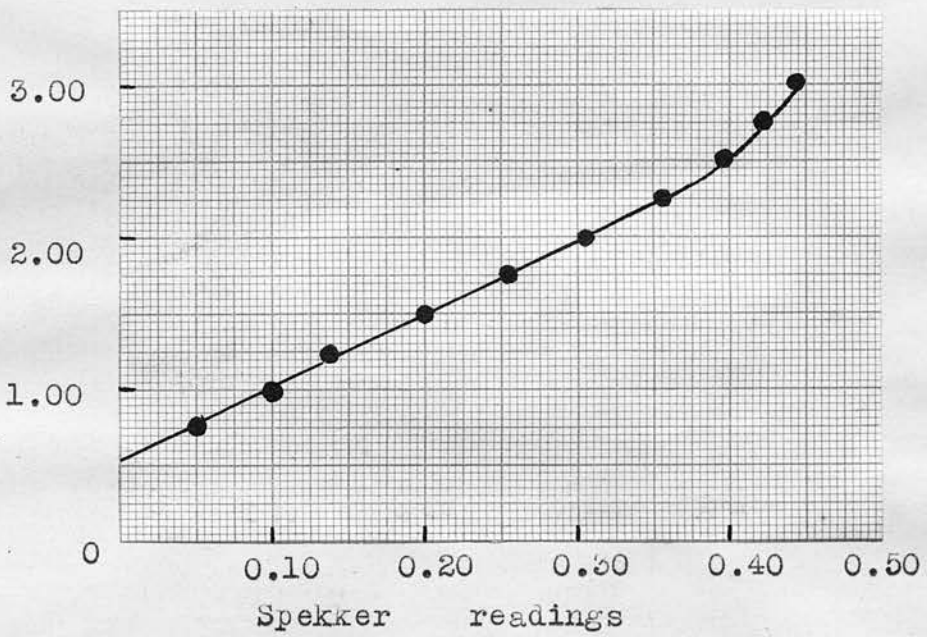
Mg. Sulphacetamide Added.	Mg. Sulphacetamide Recovered.	Percentage Recovery.
0.10	0.983	98.3
0.13	0.126	96.9
0.15	0.148	98.7
0.19	0.188	99.0

Table 2. Recovery of added amounts of sulphacetamide from guinea pig skin, by the method of Strakosch and Clark.

Table 2 shows the recoveries of added amounts of sulphacetamide to skin, extracted by heating with 2N hydrochloric acid. The results show that the method is satisfactory.

(b) Aromatic amidines. Figure 7 shows the Spekker calibration curve for V.187, estimated by Fuller's method. Over concentrations ranging from 0.75 mg. per 100 ml. to 2.25 mg. per 100 ml. the line is straight with a maximum error of less than 3%. The line cannot be extrapolated beyond that range, probably due to an insufficiency of glycerol in the reaction.

Figure 7



Spekker calibration curve for the estimation of V.187.

Mg. V.187 added.	Mg. V187 recovered	Percentage Recovery.
0.10	0.98	98%
0.12	0.116	96%
0.14	0.142	102%
0.16	0.160	100%
0.18	0.174	97%
0.20	0.198	99%

Table 3 Recovery of V.187, added to rabbit conjunctival tissue.

Table 3 shows that the method of extraction described is satisfactory.

2. The effect of wetting agents on the penetration of sodium sulphacetamide and V.187 hydrochloride into the ocular tissues.

(a) Application of drops of sodium sulphacetamide.

Table 4 shows the concentrations of sulphacetamide found in the ocular tissues, fifteen minutes, one hour, and three hours after the application of drops of 10% (W/V) sodium sulphacetamide to one eye of a rabbit, and 10 per cent (W/V) sodium sulphacetamide plus 0.1 per cent dodecyl sodium sulphate to the other eye of the same rabbit. Generally, the concentration of the drug in the tissues of the eye to which the drug is applied with the wetting agent are greater than those for the eye to which the drug is applied alone. That is always so, for the cornea, iris, and aqueous, fifteen minutes and one hour after the application of the drugs, but the results for these tissues three hours after application are not significantly different. In the sclera, the concentrations are higher when the drug is applied with the wetting agent, fifteen minutes and one hour after application, with one exception (rabbit 1458A), but the results for the conjunctiva do not at any time indicate any definite increase. In Figure 8 the means of the concentrations of sulphacetamide in the tissues are plotted against the time after application. The aqueous differs from the tissues in that the drug concentration reaches a maximum one hour after application, compared with 15 minutes in the tissues; this difference is probably due to the time taken for the drug to diffuse through the cornea into the aqueous. Taking 10 mg. per

(see p. 50)

TABLE 4.

Time After Application	Rabbit No.		Concentration of Sulphacetamide (Mg. per 100 gm.)				
			Conjunctiva	Aqueous	Cornea	Iris	Sclera
15 Mins	1454A	S	52.0	1.7	7.2	-	4.3
		S+D	36.4	5.3	18.0	4.7	32.0
	1458A	S	20.0	0.36	12.2	1.2	6.4
		S+D	36.0	2.6	19.5	5.0	3.3
	1464A	S	30.5	0.13	8.6	5.8	5.8
		S+D	18.8	0.52	17.5	11.5	11.5
	1465A	S	16.4	0.07	11.9	4.7	-
		S+D	31.2	0.73	14.6	5.0	2.4
	1466A	S	22.4	0.51	7.0	6.3	4.2
		S+D	65.3	2.1	23.3	15.8	25.9
	Mean	S	28.2	0.55	9.4	3.6	5.2
		S+D	37.5	2.25	18.6	8.4	13.0
1 Hour	1476A	S	19.1	1.6	13.2	6.4	5.5
		S+D	14.4	6.2	18.3	13.2	15.1
	1477A	S	20.0	0.39	5.8	-	2.3
		S+D	17.2	2.8	23.2	7.7	5.0
	1479A	S	7.0	0.24	4.2	<0.7	<0.7
		S+D	10.5	1.59	9.6	<0.7	6.3
	1481A	S	6.3	0.38	3.0	2.0	8.2
		S+D	18.2	2.29	13.2	12.7	18.0
	1482A	S	19.9	0.92	6.4	3.6	<0.5
		S+D	10.8	2.02	15.6	2.2	-
	Mean	S	14.4	0.70	6.5	3.2	3.1
		S+D	14.2	2.98	15.9	7.3	11.1

Table 4 cont. next page.

Time After Application	Rabbit No.		Concentration of Sulphacetamide (Mg. per 100 gm.)				
			Conjunctiva	Aqueous	Cornea	Iris	Sclera
3 Hours	1527A	S	8.1	-	8.8	3.9	1.7
		S+D	2.4	-	6.3	3.0	<0.5
	1528A	S	9.8	1.20	6.0	4.2	<0.5
		S+D	15.8	2.03	8.5	3.8	<0.5
	1529A	S	12.4	<0.01	4.7	<0.5	5.8
		S+D	15.7	1.15	6.2	1.6	5.0
	1531A	S	13.5	<0.01	7.9	<0.5	<0.5
		S+D	8.9	1.90	6.5	5.7	<0.5
	1533A	S	1.4	<0.26	<0.5	<0.5	<0.5
		S+D	13.2	0.30	2.1	1.2	1.6
	Mean	S	9.0	0.38	5.6	0.7	1.5
		S+D	11.2	1.35	5.9	3.1	1.3

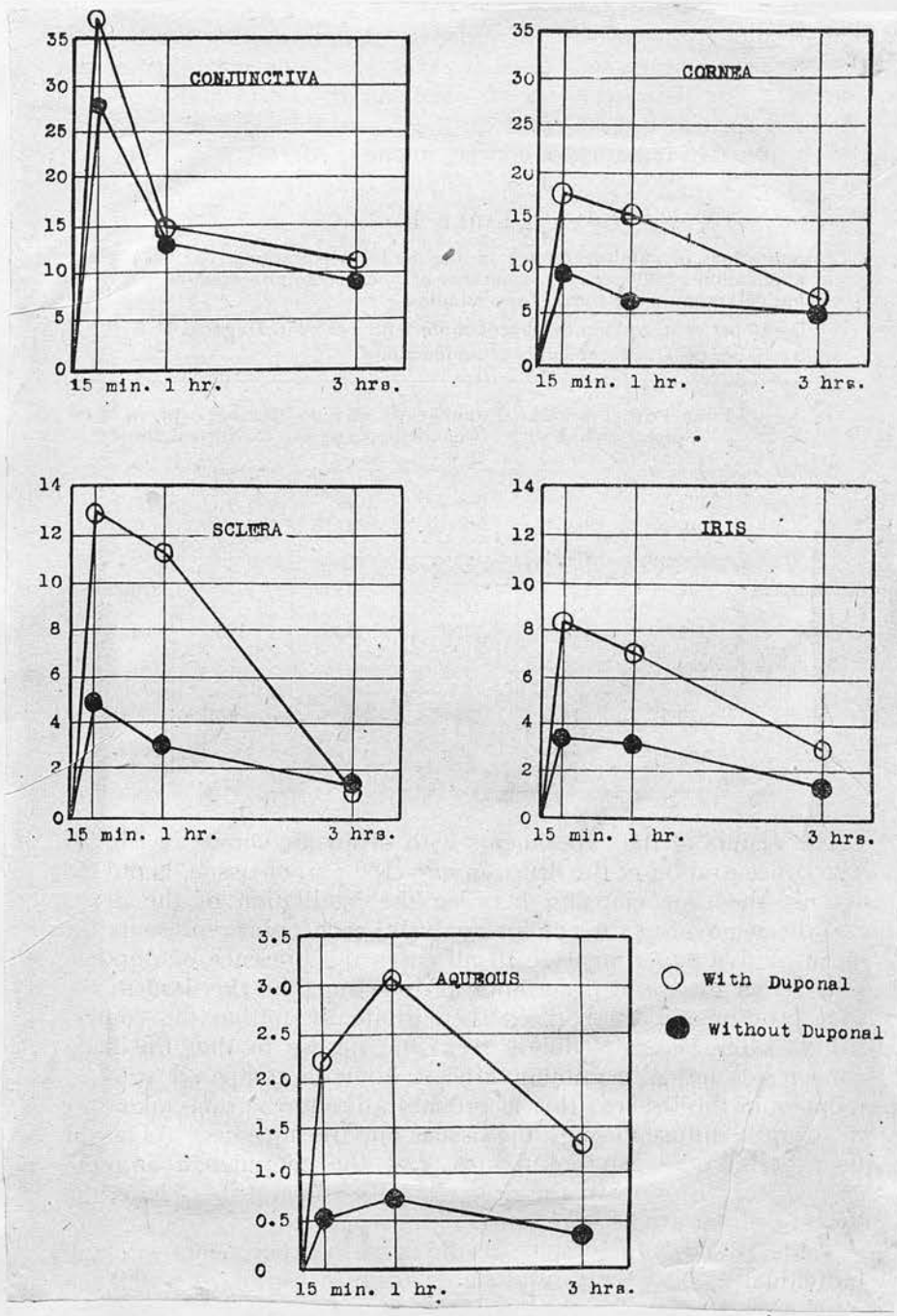
Table 4

Concentrations of sulphacetamide in the ocular tissues after the application of 0.1 ml. of 10 per cent (W/V) sodium sulphacetamide with and without 0.1 per cent (W/V) dodecyl sodium sulphate to the conjunctival sac.

S - Sodium Sulphacetamide

S+D - Sodium Sulphacetamide plus dodecyl sodium sulphate.

Figure 8



Time after application of drops.

The distribution of sulphacetamide in the ocular tissues after a single application of drops of sodium sulphacetamide, with and without dodecyl sodium sulphate (Duponal)

100 gm. as a concentration of sulphacetamide likely to be effective against severe infections, and 5 mg. per 100 gm. as effective against moderate infections, Figure 8 shows that (a) in the conjunctiva these levels are maintained for three hours, both with and without the wetting agent, (b) in the cornea the higher level is maintained for two hours, when the drug is applied with the wetting agent, ~~as~~ compared with fifteen minutes when applied alone; 5 mg. per 100 gm. is maintained for three hours in both cases, (c) in the sclera, concentrations effective against severe infections, which are not reached when the drug is applied alone, are maintained for one hour after the application of the drug plus the wetting agent; the period during which levels effective against moderate infections are maintained is increased from fifteen minutes to over two hours, (d) in the iris, 5 mg. per 100 gm. which is never reached without the wetting agent/is maintained with it on the average, for about two hours, (e) therapeutic levels are not reached in the aqueous either with or without the wetting agent.

Table 5 shows the results of three experiments in which 10% (W/V) sodium sulphacetamide with an without 0.1% (W/V) dodecyl sodium sulphate, was applied in drops to rabbit's eyes at intervals of 1 hour, for six hours. Again, in every case, the wetting agent increased the concentration of the drug in all the tissues (including the conjunctiva) in all

the rabbits, though in the cornea and iris the differences are so slight that they are of doubtful significance.

Rabbit No.		Concentration of Sulphaectamide (mg. per 100 gm) Conjunctiva	Aqueous	Cornea	Iris	Sclera
1579A	S	30.0	1.2	9.8	6.7	9.3
	S+D	40.6	4.9	14.6	9.5	17.0
1581A	S	48.6	-	20.0	10.8	11.5
	S+D	69.7	-	26.9	11.5	42.0
1583	S	21. 1	0.73	22.5	10.8	14.3
	S+D	63.5	4.3	23.3	17.7	18.1
Mean	S	33.2	0.96	17.4	9.4	11.7
	S+D	57.9	4.6	21.6	12.9	25.2

Table 5 Concentrations of sulphacetamide in the ocular tissues after (six) applications of drops of 10 per cent (W/V) Sodium Sulphacetamide with and without 0.1 per cent (W/V) dodecyl sulphate to the conjunctival sac at hourly intervals.

(b) Continuous Application of sodium sulphacetamide to the eye.

The results of experiments in which 10% (W/V) sodium sulphacetamide with and without 0.1% (W/V) dodecyl sodium sulphate was applied continuously for six minutes to the eyes of rabbits (see p.) are shown on Table. 6. Results are shown for experiments on normal eyes in vivo, normal eyes in vitro, and on eyes with denuded cornea in vivo. In normal eyes the incorporation of the wetting agent in the drug solution, without exception,

increases the concentration of the drug in the ocular tissues. There is no significant difference between the results for the experiments *in vivo* and *in vitro*, except in the case of the conjunctiva; in that tissue there are a higher concentrations in the isolated eyes. The difference is probably due to the uptake of the drug into the circulation, *in vivo*, through the blood vessels of the conjunctiva. The results for eyes with denuded cornea show that the removal of the epithelium leads to a great increase in the concentration of sulphacetamide in the tissues. The increase is particularly marked in the cornea, aqueous and iris, and less marked in the conjunctiva and sclera. The wetting agent has no effect on the penetration of the drug into denuded cornea, but its effect on the concentration of the drugs in the conjunctiva remains unchanged.

See over for table 6.

Condition.	Rabbit No.		Concentration of Sulphacetamide (mg per 100 gm) Conjunctiva	Aqueous	Cornea	Iris	Sclera.
Normal Cornea In Vivo	1406A	S	231	-	182	-	-
		S+D	225	49.4	374	-	-
	1409A	S	80	7.1	144	13.5	40.7
		S+D	870	10.0	278	31.0	191.
	1411A	S	185	7.4	212	12.2	42.9
		S+D	328	61.6	547	35.0	182.
	1419A	S	56.8	4.9	160	12.4	4.2
		S+D	121	-	204	13.2	27.8
	1426A	S	96	-	225	13.0	46.0
		S+D	485	15.9	397	18.5	107.
	1432A	S	114	6.3	241	19.0	13.0
		S+D	219	8.8	296	26.6	67.0
	Mean	S	127	6.4	191	14.0	29.3
		S+D	391	29.1	349	24.7	121
Normal Cornea In Vitro	1551A	S	643	4.1	121	16.8	44. 6
		S+D	1710	8.2	369	23.9	335
	1553A	S	145	7.6	129	12.2	46. 9
		S+D	994	17.2	439	31.3	127
	1557A	S	412	2.8	185	10.6	88. 0
		S+D	1290	22.4	303	63.5	145
	Mean	S	400	4.8	145	13.2	59. 1
		S+D	1427	15.9	370	39.6	203.
Denuded Cornea In Vivo	1647A	S	269	279	1050	196	100
		S+D	343	274	1110	185	112
	1648A	S	447	412	1300	297	220
		S+D	545	283	1490	216	108
	1649A	S	342	675	1230	220	219
		S+D	686	452	1110	184	132
	Mean	S	353	455	1370	238	179
		S+D	525	336	1240	195	117

Table 6. Concentrations of Sulphacetamide in the ocular tissues after the continuous application of 10 per cent (W/V) sodium sulphacetamide with and without 0.1 per cent (W/V) dodecyl sodium sulphate for six minutes.

S Sodium Sulphacetamide.

S + D Sodium Sulphacetamide plus dodecyl sodium sulphate.

(c) Continuous Application of sodium Sulphacetamide to isolated ocular tissues.

The results for experiments in which 10% (W/V) sodium sulphacetamide with and without 0.1% (W/V) dodecyl sodium sulphate, was applied continuously for 15 minutes to isolated ocular tissues (see p. 13) are shown on Table 7. This method gives more constant results than the other methods of demonstrating the action of the wetting agent. The results show that the action is confined to the cornea with the epithelium intact, and does not occur with denuded corneae or with sclera (both of which differ from intact cornea in that they do not possess an epithelium.).

See Over for Table 7

Tissue.	Rabbit No.	Concentration of Sulphacetamide.					
		With Wetting Agent.			Without Wetting Agent.		
		Tissue	Anterior Chamber Soln. 5mins	Anterior Chamber Soln. 15mins	Tissue	Anterior Chamber Soln. 5mins	Anterior Chamber Soln. 15mins.
Normal Cornea	1509A	844	0.5	22.3	229	0.4	9.2
	1626A	789	2.5	37.4	464	0.6	9.7
	1631A	777	0.8	28.8	383	1.6	6.0
	Mean	803	1.3	29.5	357	0.9	8.3
Denuded Cornea	1558A	3160	35.6	201	3700	41.3	239
	1559A	3480	14.5	196	-	15.0	-
	1560A	3680	25.3	203	3540	20.3	181
	Mean	3440	25.1	200	3620	25.2	210
Sclera	1510A	4020	32	165	3310	57	235
	1512A	2950	25	-	3330	36	-
	1514A	2740	26	139	4330	37	218
	Mean	3240	28	152	3660	43	226

Table 7 Concentrations of Sulphacetamide penetrating into and through ocular tissues after the application of 10 per cent (W/V) sodium Sulphacetamide with and without 0.1 per cent (W/V) dodecyl sodium sulphate for 15 mins *in vitro*

(d) Comparison of different concentrations of various wetting agents in their effectiveness in increasing penetration of sodium sulphacetamide into the eye. Table 8 shows the results for 35 experiments on the effect of wetting agents on the absorption of 10 percent sodium sulphacetamide by the cornea; in the in vitro experiments, the drug was applied for 15 minutes, while in the in vivo experiments it was applied for 6 minutes. The following wetting agents were used, dodecyl sodium sulphate, ammonium lorol, aerosol OT (di sodium dioctyl succinate), lissapol N, and C 60799. The last two wetting agents were supplied by I.C.I., and are non-ionic and therefore form stable solutions with substances which form insoluble salts with other wetting agents, or which tend to coagulate the wetting agent.

See over for Table 8.

TABLE 8

Wetting Agent	Concentration gms. percent	Experiment	Number of Experiments.	Conc. of Sulphacetamide (mg. percent)			
				Cornea		Anterior Fluid	
				W.E.	Control	W.E.	Control
Dodecyl sodium sulphate.....	0.1	In vitro	3	803	357	29.5	8.3
"	0.5	"	2	1300	342	38.7	8.3
"	1.0	"	2	1915	315	54.4	6.9
"	0.1	In vivo	6	349	191	29.1	6.4
"	1.0	"	1	860	200	77.2	16.1
Ammonium Lorol	1.0	In vitro	2	886	478	17.6	8.9
Aerosol OT	0.1	"	1	434	-	6.9	-
"	0.2	"	1	451	449	8.5	7.6
"	1.0	"	3	398	365	10.2	11.6
"	1.0	In vivo	4	389	108	32.3.	8.0
Lissapol N.	0.1	In vitro	1	430	372	4.1	3.7
"	0.25	"	1	603	346	10.3	4.0
"	0.5	"	1	1000	366	7.3.	5.0
"	1.0	"	2	1050	295	19.8	4.7
"	0.1	In vivo	1	114	186	4.2	8.7
"	1.0	"	1	601	155	84.9	13.3
C. 60799	1.0	In vitro	2	288	323	2.3	2.6
"	1.0	In vivo	1	48.8	61.9	3.3	5.5.

The effect of different concentrations of wetting agents on the penetration of 10 percent sodium sulphacetamide into and through the cornea.

The results on Table 8 show that dodecyl sodium sulphate is the most effective wetting agent both in vivo and in vitro; lissapol N is the second most effective wetting agent tested. The minimum effective concentration of lissapol is in the region of 0. 25 percent which gives an increase of 75% in drug concentration in the cornea, while 0.1 percent dodecyl sodium sulphate causes an increase of 125% (when applied for 15 minutes in vitro). C 60799 is totally ineffective both in vivo and in vitro. (1943) Aerosol OT has been reported by Bellows and Gutman to be very effective; ~~but~~ it does not have any effect in vitro, but in vivo, in a concentration of 1 percent it causes an increase of 260%, compared with 330% for 1 percent dodecyl sodium sulphate, and 288% for 1 percent lissapol N. Detailed results of experiments are shown in Table 9 in which the drug, with Aerosol OT was applied to the eyes of rabbits in vivo, and under the same conditions to the eyes of dead and cold rabbits.

See over for Table 9

Condition.	Rabbit No.		Concentration of Sulphacetamide (Mgs./100 gm.)				
			Conjunctiva	Aqueous	Cornea	Iris	Sclera
In vivo	1682A	S	167	10. 9	133	13.6	59.1
		S+A	286	78. 2	802	60.2	123
	1685A	S	121	5.04	45.4	1.0	30.4
		S+A	335	32. 8	265.	27.3	94.0
	1690A	S	194	7. 8	138	17.3	70.2
		S+A	—	10. 1	173	27.8	71.3
	1699A	S	59.3	8. 2	117	12.2	7.5
		S+A	627	8. 3	316	39.4	110
	Mean	S	135	8. 0	108.3	11.0	42.0
		S+A	416	32.3	387	38.9	99.8
In vitro	1700A	S	58.6	3. 7	854	11.3	10.0
		S+A	840	4. 7	155	24.4	49.3
	1701A	S	1350	3. 2	116	8. 6	75.9
		S+A	496	3. 9	141	9. 4	16.5
	Mean	S	704	3. 5	100.2	9. 9	42. 9
		S+A	668	4. 3	148	16. 9	30. 4

S 10 per cent (W/V) sodium sulphacetamide.

S+A 10 per cent (W/V) sodium sulphacetamide plus 1 per cent (W/V) Aerosol OT.

Table 9. Concentration of sulphacetamide in the ocular tissues after the continuous application in vivo and in vitro of 10 percent (W/V) sodium sulphacetamide with and without 1 per cent (W/V) Aerosol OT, to the eye of a rabbit.

This difference in the action of aerosol OT *in vivo* and *in vitro* may be due to temperature differences. In all cases where the wetting agents caused a great increase in absorption of the drug, the cornea was found to be oedematous and covered with a mucoid discharge; this was found to be the case with 1 percent aerosol *in vivo*, but *not in vitro*. The significance of these results are discussed in a subsequent section. The increase in the absorption of the drug in the presence of the wetting agents is roughly proportional to the concentration of the wetting agent, within the range of concentrations used.

See over for Table 10

Tissue etc.	Concentration of Sulphacetamide (mgs. per cent)			
	Lissapol N.(0.5%)		Dodecyl Sod.Sulphate (0.5%)	
	24 hrs old	Fresh	24 hrs old	Fresh
Cornea	668	695	653	1240
Anterior Fluid	5.5	5.7	46.1	19.0

TABLE 10.

The effect of age on the absorption of sulphacetamide by the cornea, from solutions of 10 per cent sodium sulphacetamide with 0.5% dodecyl sodium sulphate and 0.5 per cent lissapol N.

In contrast to dodecyl sodium sulphate, which coagulates, lissapol N remains permanently in solution in the presence of 10 per cent sodium sulphacetamide, and as shown on Table 10 it does not lose its activity in 24 hours as does dodecyl sodium sulphate.

Rabbit No.	Wetting Agent	Concentration of Sulphacetamide mg./100 gm.				
		Conjunctiva	Aqueous	Cornea	Iris	Sclera
1772A	0.5% Lissapol	47.9	1.2	19.8	5.4	10.3
	0.1% Dodecyl Sodium Sulphate	60.0	1.2	11.7	4.4	11.6
1775	0.5% Lissapol	27.8	2.0	22.4	0.3	1.0
	0.1% Dodecyl Sodium Sulphate	36.6	1.7	17.3	0.25	0.8
Mean	0.5% Lissapol	37.9	1.6	21.1	2.9	5.5
	0.1 Dodecyl Sodium Sulphate	48.3	1.5	14.5	2.3	6.2

TABLE 11

Concentrations of sulphacetamide in the ocular tissues 15 minutes after the application of 0.1 mil of 10 per cent (W/V) sodium sulphacetamide with either 0.1 per cent (W/V) dodecyl sodium sulphate or 0.5 per cent (W/V) lissapol N.

Table 11 shows that when 0.5 percent lissapol N with 10 per cent sodium sulphacetamide is applied in drops to the conjunctival sac the absorption of the drug by the ocular tissues is approximately the same as when 0.1 per cent dodecyl sodium sulphate plus 10 per cent sodium sulphacetamide is used.

(e) The Penetration of V.187 into the eye.

Table 12 shows the results of experiments in which 10% (W/V) V.187 hydrochloride was applied for 15 minutes to the eye of a rabbit *in vivo*, and either 10% (W/V) V.187 hydrochloride plus 0.5% (W/V) lissapol N or 10% (W/V) sodium sulphacetamide to the other eye.

Rabbit No.	Drug	Concentration of drug mg / 100 gm. Conjunctiva	Cornea
1757A	(V.187 (10%)	219	503
	(1% Lissapol		
	V.187	101	184
1758A	(V.187		
	(1% Lissapol	180	412
	V.187	114	167
1760A	10% V.187	107	281
	10% Sod.Sulphacetamide	213	400
1762A	10% V.187	90	<100
	10% Sod.Sulphacetamide	186	321

TABLE 12

Concentrations of 10 per cent (W/V) V.187 in the ocular tissues after continuous application for 15 minutes with and without a wetting agent, compared with concentrations of sulphacetamide after the application of 10 per cent (W/V) sodium sulphacetamide for 15 minutes.

The penetration of sulphacetamide into the eye is greater than that of V.187; the sulphacetamide concentrations in the ocular tissues are approximately twice the concentrations of V.187. Application of V.187 hydrochloride with the wetting agent, enables the drug to penetrate more readily into the eye, and the concentrations in the ocular tissues are increased.

(f) The Diffusion of Sodium Sulphacetamide into gels.

The results of experiments to determine whether the action of the wetting agents in increasing the penetration of drugs into the eye was due to an increase in the rate of diffusion of the drug, are shown on Tables 13a and 13b. The results of the experiments designed to measure the diffusion constant of the drug in gelatin gels are shown on Table 13a (i.e. in which the drugs diffused out the gels), and the results for experiments in which the drug diffused into the gel are shown on Table 13b.

See over for Table 13a.

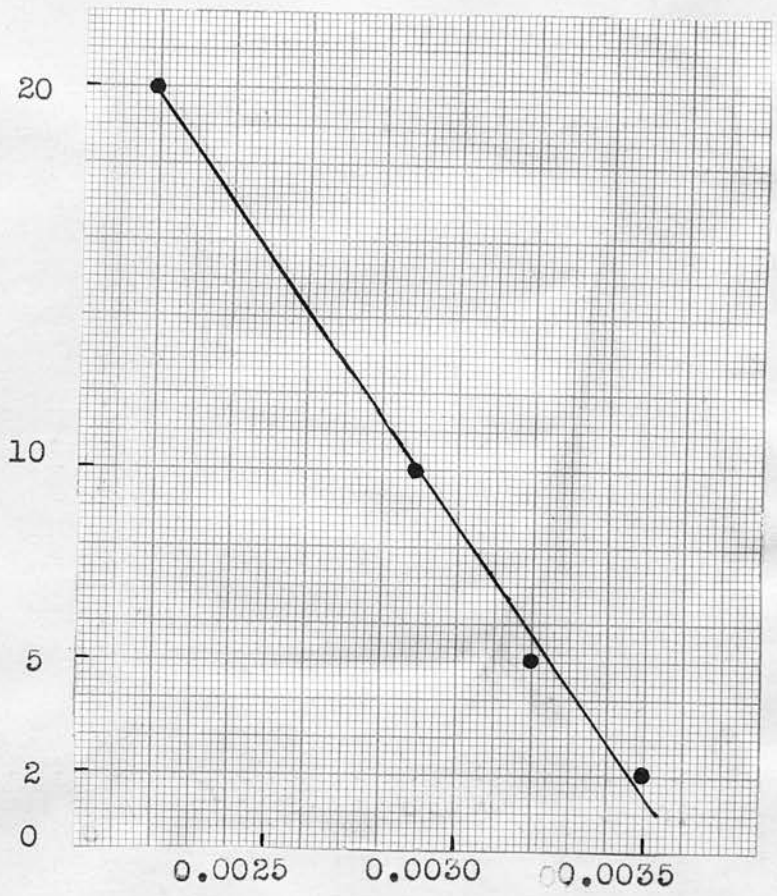
Concentration of Gel gms/100 ml.	Contents of Gel	Wt. of Sodium Sulphacetamide diffusing out. mg.	Ht. of 10ml. Saline in tube. cm.	Area of Cross Section of tube cm^2.	Mean Concentration Difference am/sulphacetamide/10ml.	Diffusion Constant.
2	S	31.5	2.0	5.0	2.842	0.0035
	S	31.7	2.0	5.0	2.841	0.0036
	S + D	31.2	2.0	5.0	2.844	0.0035
	S + D	31.4	2.0	5.0	2.843	0.0035
5	S	30.0	2.1	4.74	2.850	0.0034
	S	29.1	1.9	5.26	2.855	0.0031
	S + D	29.1	2.0	5.0	2.855	0.0033
	S + D	29.5	1.9	5.26	2.852	0.0031
10	S	26.8	2.0	5.0	2.866	0.0030
	S	26.6	1.9	5.26	2.867	0.0028
	S + D	26.9	2.0	5.0	2.865	0.0030
	S + D	26.6	1.9	5.26	2.867	0.0029
20	S	21.5	1.9	5.26	2.892	0.0023
	S	22.7	1.8	5.55	2.886	0.0023
	S + D	22.7	1.9	5.26	2.886	0.0024
	S + D	22.0	1.9	5.26	2.890	0.0023

Table 13a. Diffusion constants of sodium sulphacetamide through gelatin gels.

S - 3% (W/V) sodium sulphacetamide.

S+D - - - - - - - - - - - - - - - - - - 0.1%(W/V) dodecyl sodium sulphate.

Figure 9



Showing the relationship between the diffusion constant for sodium sulphacetamide and the concentration of gelatin in the gel.

In both cases the wetting agent did not show any effect on the diffusion of the drug.

Solution	Diameter of tube (D)	Total mg. Sulphacetimide in gel. (W).	W/D
S	2.4 cm.	405 mg.	169
S	2.3 cm.	384 mg.	166
S+D	2.4 cm.	405 mg.	169
S+D	2.3 cm.	377 mg.	164

S - 10% (W/V) sodium sulphacetamide

S+D - - - - - - - - - - - - - - - - - - 0.1% dodecyl sodium sulphate.

TABLE 13b.

Diffusion of sulphacetamide into gels.

Figure 9 shows that the diffusion constant is inversely proportional to the concentration of gelatin in the gel.

(g) Interfacial tensions of sodium sulphacetamide and wetting agent solution.

Table 14 shows the results of the experiments to determine whether there is any relation between the lowering of the surface tension of the drug solutions, and the increased penetration of the drugs into the cornea, in the presence of wetting agents. No relationship is apparent from the results. There are only very slight differences between the results for the wetting agents alone in water or with sodium

sulphacetamide, for drops formed at both air /water and paraffin/ water interfaces.

Concentration of Lissapol (gms. per cent)	Air/Water		Paraffin/Water	
	Wt.of 10 drops(mgs.)		Vol.of 10 drops(ml.	
	In water	In 10% Sod. sulphaceta-mide.	In water	In 10% Sod. Sulphsceta-mide.
0	526	503	3.50	1.79
0.1	200	203	0.105	0.095
0.25	201	204	0.088	0.071
0.5	201	205	0.076	0.063
1.0	201	205	0.071	0.060

TABLE 14a.

Interfacial tensions at air/water and paraffin/ water interfaces of Lissapol N solutions in water and in 10 per cent sodium sulphacetamide in water.

With drops of lissapol N solutions formed at air/ water interfaces the weight of the drops are considerably lower than the weight of drops of water, but do not vary significantly between concentrations 0.1 to 1.0% (W/V) lissapol. Similarly, the volumes of drops of wetting solutions formed at a paraffin/water interface are considerably lower than those of water, and do not change significantly when the concentration of the wetting agent is increased.

Wetting Agent		Percentage increases in absorption by cornea	IT. Water/Paraffin Vol. of 10 drops.
NIL		0	1.79
Lissapol N	0.1%	14	0.095
"	0.25%	74	0.071
"	0.5%	170	0.063
"	1.0%	290	0.060
Dodecyl Sodium Sulphate	0.1%	125	0.148
"	0.25%	-	0.138
"	0.5%	300	0.149
"	1.0%	510	0.144
Aerosol OT	1.0%	10	0.044

TABLE 14b.

Effect of wetting agents on the interfacial tension at a water/paraffin interface and the percentage increase in the absorption by the cornea of 10 per cent sodium Sulphacetamide.

Thus there is practically no difference in the drop volume of dodecyl sodium sulphate solutions in 10% (W/V) sodium sulphacetamide over a range of concentration of the wetting agent, for which the range of the corresponding increase in the concentration of the drug found in the cornea, is 125 per cent to 510 per cent, after application of the solution for 15 minutes in vitro. Although dodecyl sodium sulphate is the most effective in increasing the absorption of the drug by the cornea, and aerosol one of the least effective, the latter produces lower interfacial tensions than the former. Thus no relation between the interfacial tension with air and paraffin of wetting agent solutions in water and in 10 per cent sodium sulphacetamide in water and the effect of the wetting agents on the absorption of the drug by the cornea could be found.

(h) The action of wetting agents on corneal epithelium. Attempts to remove the epithelium from corneae which had been treated with wetting agents in 10 per cent sodium sulphacetamide were unsuccessful, and suggest that the epithelium is removed either partly or completely, through the action of the wetting agents. The wetting agent alone and with the drug was applied to the eyes of rabbits through a funnel for 15 minutes, after which the cornea was stained with fluorescein.

This showed that 0.1 per cent dodecyl sodium sulphate both with and without the drug caused damage to the epithelium over the whole of the cornea. With 0.05 per cent and with 0.01 per cent solutions, the staining was very slight and not much greater than that caused by application of saline or water, and may have been caused by the funnel.

Experiments were also carried out applying 3 drops of 0.1 per cent and 1 per cent dodecyl sodium sulphate in water to the eyes of a rabbit at hourly intervals for six hours, after which the eyes were carefully examined by Dr. A.A.B. Scott, using a slit lamp ophthalmoscope. In the eyes to which 0.1 per cent dodecyl sodium sulphate was applied, no abnormalities were seen except that there was punctate staining confined to the surface. In the eye treated with 1 per cent dodecyl sodium sulphate there was an appearance of oedema of the epithelium and after fluorescein the cornea was covered with punctate staining confined to the anterior surface, but much more marked than in the case of the eye treated with 0.1 per cent dodecyl sodium sulphate. The application of drops of 1 per cent lissapol N in saline at hourly intervals for six hours, resulted in slight discharge from the eye and very slight conjunctival oedema. By the next morning there was still slight oedema but no discharge and there was very slight staining at the centre of the cornea. Two successive hourly

applications of drops of 0.5 per cent lissapol N in saline to the eyes of ~~the~~ a rabbit resulted in no abnormalities or staining.

These results indicate that prolonged application of high concentrations of wetting agents to the cornea results in the removal of the corneal epithelium, which is the cause of the mucoid discharge on the surface of the cornea referred to in a previous section. When the wetting agent is applied in drops, after many applications slight damage is caused to the surface of the epithelium, but one or two applications do not cause any perceptible damage. When wetting agents increase the absorption of drugs by the cornea after a single application of drops to the conjunctival sac, the entry of the drug is facilitated by an imperceptible derangement of the epithelium. Wetting agents should not therefore, be used repeatedly. Since the site of action of the wetting agents is in the cornea, the absorption of all water soluble drugs by the cornea should be increased.

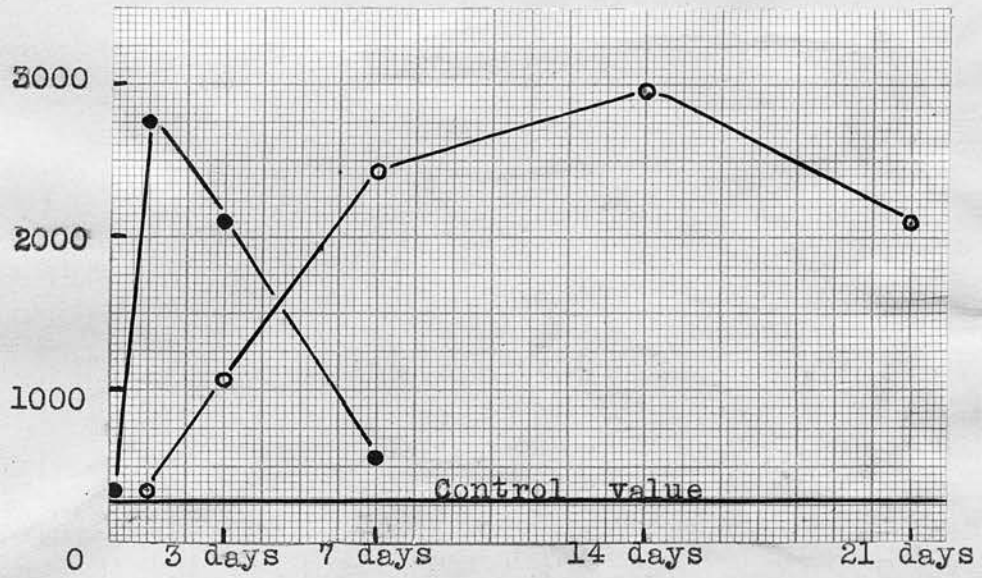
3. The Penetration of sodium sulphacetamide into the tissues of eyes damaged with chemical warfare agents.

The effects of contamination with H and NH on the penetration of sodium sulphacetamide into the cornea in vitro, are shown on Figure 10 and Table 15. In these experiments, only one eye in each rabbit was treated with the chemical warfare agent, the other being used as a control. The control value shown on Figure 10 is the mean of all the control experiments performed.

Time After Contamination	Concentration of Sulphacetamide			
	H.		NH.	
	Cornea	Anterior Chamber	Cornea	Anterior Chamber
0	388	9.1	388	9.1
1 hr.	471	14.2	-	-
	332	5.4		-
1 day.	2030	80.9	231	3.6
	3570	138	741	17.1
	-	-	612	20.5
	-	-	436	16.3
3 days.	1650	36.2	355	10.8
	2560	60.7	280	6.2
	-	-	2050	47.8
	-	-	2640	51.2
7 days.	677	10.7	2496	58.0
	388	9.0	2380	24.3
14 days.	-	-	2340	131
	-	-	2540	91.4
21 days.	-	-	2120	44

Table 15 Penetration of Sodium Sulphacetamide in vitro into corneae contaminated with chemical warfare agents.

Figure 10



The effect of contamination with chemical warfare agents on the penetration of sodium sulphacetamide into the cornea.

The permeability of the corneae contaminated with H is not changed one hour after contamination, but is greatly increased one day later, and gradually falls to the control value, which is almost reached seven days after contamination. The results with NH contaminated eyes show that the increase in permeability does not take place as soon after contamination as with H. The results for experiments one day after contamination are not significantly different from the normal controls, and three days after contamination, in half of the experiments there is a marked increase in permeability, while in the other half the concentrations of sulphacetamide in the treated corneae are not significantly different from the control value.

Chemical Warfare Agent.	Concentration of Sulphacetamide (mg/100 gm).			
	Contaminated		Normal Control.	
	Cornea	Anterior Chamber	Cornea	Anterior Chamber
H	3590	135	3680	164
	3040	148	3020	120
NH.	2790	106	3250	152
	3840	232	3440	263

Table 16. Penetration of sodium sulphacetamide in vitro, into denuded corneae from eyes of rabbits, contaminated one day previously with chemical warfare agents.

The results on Table 16 show that the penetration of sodium sulphacetamide into denuded corneae is not affected by the contamination of the eye with the chemical warfare agents.

Table 17 shows the concentrations of sulphacetamide in the ocular tissues, fifteen minutes after a single application of 0.1 ml. of 10 per cent (W/V) sodium sulphacetamide, with and without 0.1 per cent (W/V) dodecyl sodium sulphate, to the eyes of rabbits which had been contaminated one day previously with H. The results for the contaminated eyes, except in the case of the aqueous, are not significantly different from the results obtained when sodium sulphacetamide alone, is dropped into normal eyes (see Table 4). When the drug is applied with the wetting agent, there is no significant increase. In the aqueous, the results are significantly higher than in normal eyes, and there is also a significant increase when the drug is applied with the wetting agent.

See over for Table 17

Rabbit No.		Concentration of Sulphacetmide (mg./100 gm.)				
		Conjunctiva	Aqueous	Cornea	Iris	Sclera
1629A	S	5.3	1.6	6.0	3.7	1.6
	S+D	10.1	2.6	8.4	3.6	1.8
1630A	S	14.8	4.5	17.8	3.0	0.8
	S+D	13.8	12.0	38.2	4.5	1.5
1633A	S	37.1	1.1	9.5	-	1.4
	S+D	56.1	2.9	10.0	-	-
1634A	S	20.7	1.3	9.8	1.5	3.8
	S+D	25.2	2.75	9.0	4.0	9.9
Mean	S	19.5	2.1	14.0	2.7	1.3
	S+D	26.3	5.1	16.4	4.0	2.5

Table 17. Concentrations of Sulphacetamide in the ocular tissues, fifteen minutes after the application of 0.1 ml. 10 percent (W/V) sodium sulphacetamide, with and without 0.1 percent (W/V) dodecyl sodium sulphate, to the eyes of rabbits, contaminated one day previously with dichloroethyl sulphide (H).

S - Sodium Sulphacetamide.

S+D - Sodium Sulphacetamide plus dodecyl sodium Sulphate.

4. Intravitreous and Subconjunctival Injection.

(a) Injection of sodium sulphacetamide into normal vitreous. Table 18 shows the concentrations of sulphacetamide found in the vitreous, aqueous, cornea, iris, lens, choroid plus retina, sclera, and conjunctiva, one hr., six hrs., one day, two days, three days, and four days after the injection of 0.1 ml. of 30 per cent sodium sulphacetamide into the vitreous of normal eyes of rabbits.

The drug is rapidly taken up by all the tissues. In the vitreous, the concentration falls from the region of 1000 mgm. per 100 gm. to an average value of 2.5 mgm. per 100gm. after 4 days. In 2 cases out of four, concentrations greater than 10 mgm. per 100 gm. (i.e. of therapeutic significance) are found in the vitreous after three days, while concentrations of therapeutic value are maintained in the other tissues and fluids for approximately two days. Fig.11a. shows the log. of the concentration of sulphacetamide in the vitreous plotted against the time after injection; the log. of the concentration is inversely proportional to the time after injection. Fig.11b shows the log. of the concentration of sulphacetamide in the cornea plotted against the time after injection; the concentration rises to a maximum after 6 hours, and then falls at a rate such that the log. of the concentration is inversely proportional to the time after injection.

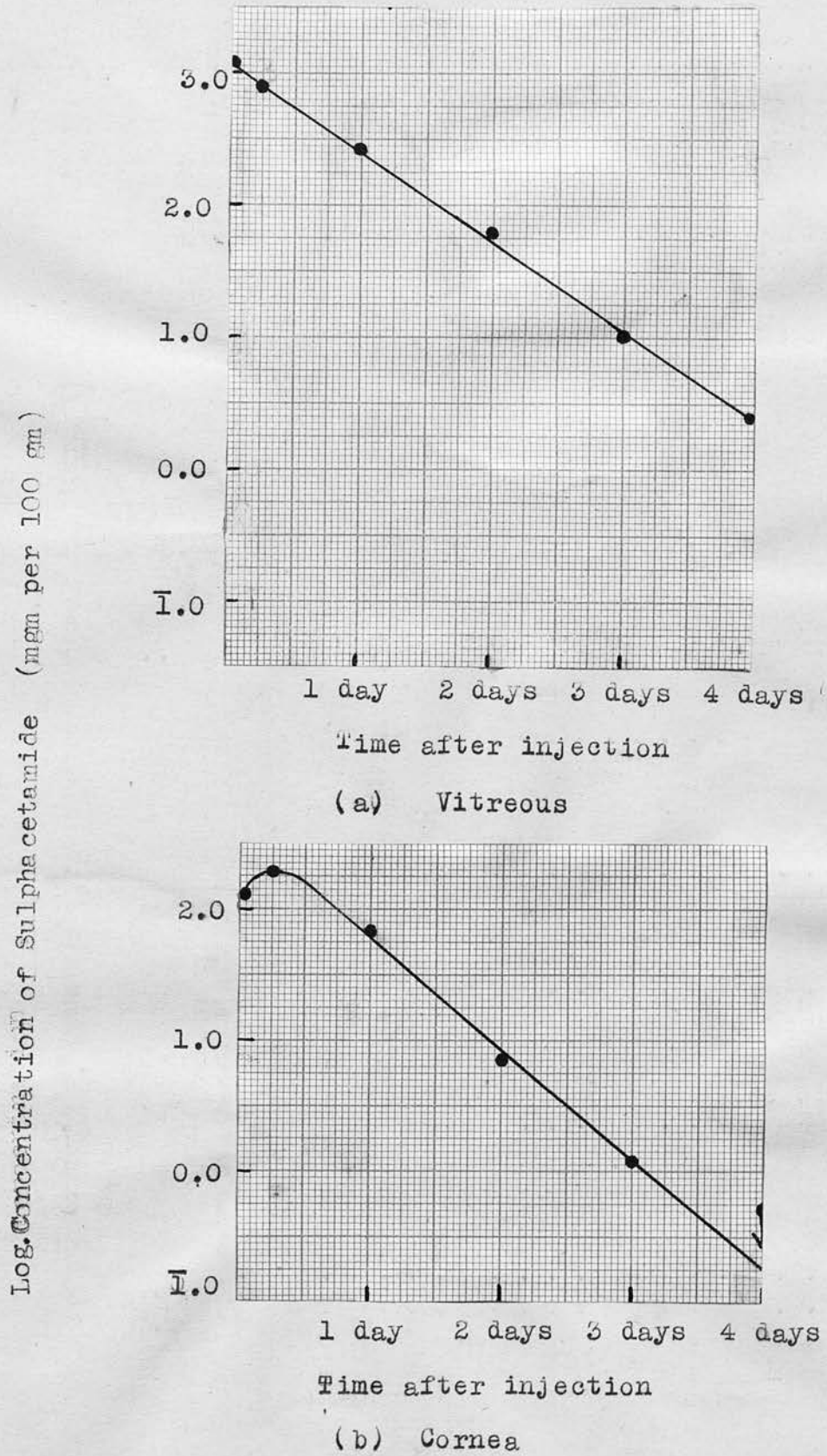
The relatively low concentrations of the drug found in the conjunctiva one hour and six hours after injection probably arise from leakage through the injection puncture.

See over for Table 18.

Time	Rabbit No.	Mgs. Sulphacetamide /100 gms.							
		Vitreous	Aqueous	Cornea	Iris	Lens	Choroid and Retina	Sclera	Conjunctiva
1 hr	1896 R.E.	1300	232	102	122	21.1	290	213	-
	1898A "	1300	69.3	31.6	183	28.3	250	212	-
	1910A (R.E.	960	268	177	308	-	391	149	1.2
	(L.E.	980	342	257	440	-	351	198	8.0
6 hrs	1896 L.E.	670	-	105	138	38.0	120	115	-
	1898A "	1080	218	180	263	61.7	254	121	-
	(R.E.	550	205	187	221		202	125	7.4
	1911A (L.E.	810	275	203	253		287	197	28.9
1 day	1888 (R.E.	209	92.5	94.0	106	-	-	71.7	-
	(L.E.	353	111	84.2	12.0	-	-	43.7	-
	1900A (R.E.	310	49.9	70.0	36.6	38.5	41.0	-	-
	(L.E.	311	58.2	64.7	85.0	20.2	42.5	45.0	-
	1912A (R.E.	201	20.5	20.7	-	-	-	-	}
	(L.E.	239	63.5	80.1	74.5	-	-	-	} 0.6
2 days	1887 (R.E.	1151	18.9	11.3	19.2	-	-	38.2	-
	(L.E.	70.9	-	7.0	14.0	-	-	10.1	-
	1901A (R.E.	31.1	4.0	6.3	11.1	16.9	10.7	13.5	-
	(L.E.	25.7	3.5	7.4	12.1	13.9	9.6	7.9	-
3 days	1886 (R.E.	23.7	4.9	2.8	3.0	-	-	2.0	-
	(L.E.	13.0	0.85	1.0	1.6	-	-	1.2	-
	1902A (R.E.	1.5	0.5	1.0	1.0	15.2	-	1.0	-
	(L.E.	0.9	1.0	0.5	1.0	9.0	-	1.0	-
4 days	1903A (R.E.	3.4	0.6	0.6	-	-	-	-	-
	(L.E.	6.3	0.9	0.6	-	-	-	-	-
	1909A (R.E.	0.1	0.2	0.5	-	-	-	-	-
	(L.E.	0.5	0.2	0.5	-	-	-	-	-

Table 18. Concentrations of sulphacetamide in the ocular fluids and tissues after the intravitreous injection of 0.1 ml. 30% sodium sulphacetamide in the normal eyes of rabbits.

Figure 11



Showing the clearance of sulphacetamide from the cornea and vitreous after intravitreous injection sodium sulphacetamide.

(b) Injection of Sodium Penicillin into normal vitreous. Table 19 shows the concentrations of penicillin found in the vitreous, aqueous, and cornea, one hr., six hrs., one day, two days, and three days after the injection of 2,000 units of commercial sodium penicillin into the vitreous of normal eyes of rabbits. Table 20 shows the results for similar experiments with pure crystalline sodium penicillin (1, 600 u. per mgm.), showing also levels reached in the conjunctiva.

Time	Rabbit No.		Concentration of Penicillin (units per ml.) Vitreous	Aqueous	Cornea
1 hour	1892	RE	500-1000	20-40	16-32
6 hours	1892	LE	500-1000	5-10	8-16
1 day	1873	RE	100-300	-	-
	1873	LE	100-300	-	-
	1891	RE	100-200	3-6	2-4
		LE	200-400	6-12	4-8
	1893	RE	200-400	1-2	2-4
		LE	200-400	1-2	2-4
2 days	1874	RE	6-10	-	-
		LE	6-10	-	-
	1890	RE	⟨ 1/5	⟨1/10	⟨1/2
		LE	1-2	1/10-1/5	⟨1/2
	1894	RE	5-10	1/10-1/5	
		LE	1-2	⟨1/10	⟨1/4'
3 days	1889	RE	⟨1/10	⟨1/10	⟨1/2
		LE	⟨1/10	⟨1/10	⟨1/2
	1895	RE	1/10-1/5	⟨1/10	⟨1/4'
		LE	1/10-1/5	⟨1/10	⟨1/4'

Table 19. Concentrations of penicillin in the aqueous, vitreous and cornea, 1 hour, 6 hours, 1,2, and 3 days after the intravitreous injection of 2,000 units of commercial sodium penicillin.

' Tissues from both eyes analysed together.

Time	Rabbit No.		Concentration of Penicillin (units per ml.) Vitreous	Aqueous	Cornea	Conjunctiva
1 hour	1922	(R.E.	1000-2000	4-8	4-8	-
		(L.E.	2000-4000	2-4	8-16	-
	1934	(R.E.	500-1000	2-4	1-2	) <1/2[1]
		(L.E.	500-1000	2-4	2-4	)
		(R.E.	-	25-50	-	) 4-8[1,2]
		(L.E.				) 1/4-1/2[3]
6 hours	1921	(R.E.	500-1000	8-16	4-8	-
		(L.E.	1000-2000	2-4	4-8	-
	1935	(R.E.	1000-2000[4]	8-16	8-16	) 1/2-1[1]
		(L.E.	500-1000	8-16	8-16	)
	1939	(R.E.	-	11/2-3	-	) 1½-3[1]
		(L.E.	-	3-6	-	)
1 day	1920	(R.E.	64-128	< ¼	<1/2	-
		(L.E.				
		(	32-64	1/4-1/2	1/2-1	-
	1936	(R.E.	16-32	1/4-1/2	<1/2	) <1/4[1]
		(L.E.	32-64	1/2-1	1/2-1	)
	1940	(R.E.	-	) 1/2-1[1]		) <1/5[1]
		(L.E.	-	)		)
2 days	1919	(R.E.	1/2-1	<1/10	) <1/4[1]	-
		(L.E.	1/2-1	<1/10	)	-
	1937	(R.E.	1-2	1/10-1/5	) <1/4[1]	-
		(L.E.	1-2	1/10-1/5	)	-
3 days	1918	(R.E.	<1/10	<1/10	) <1/4[1]	
		(L.E.	<1/10	<1/10	)	
	1938A	(R.E.	<1/10	<1/10	) < 1/4[1]	
		(L.E.	<1/10	<1/10	)	

Table 20. Concentrations of penicillin in the aqueous, vitreous and cornea, 1 hour, 6 hours, 1,2,3, days after intravitreous injection of 2000 units of pure sodium penicillin.

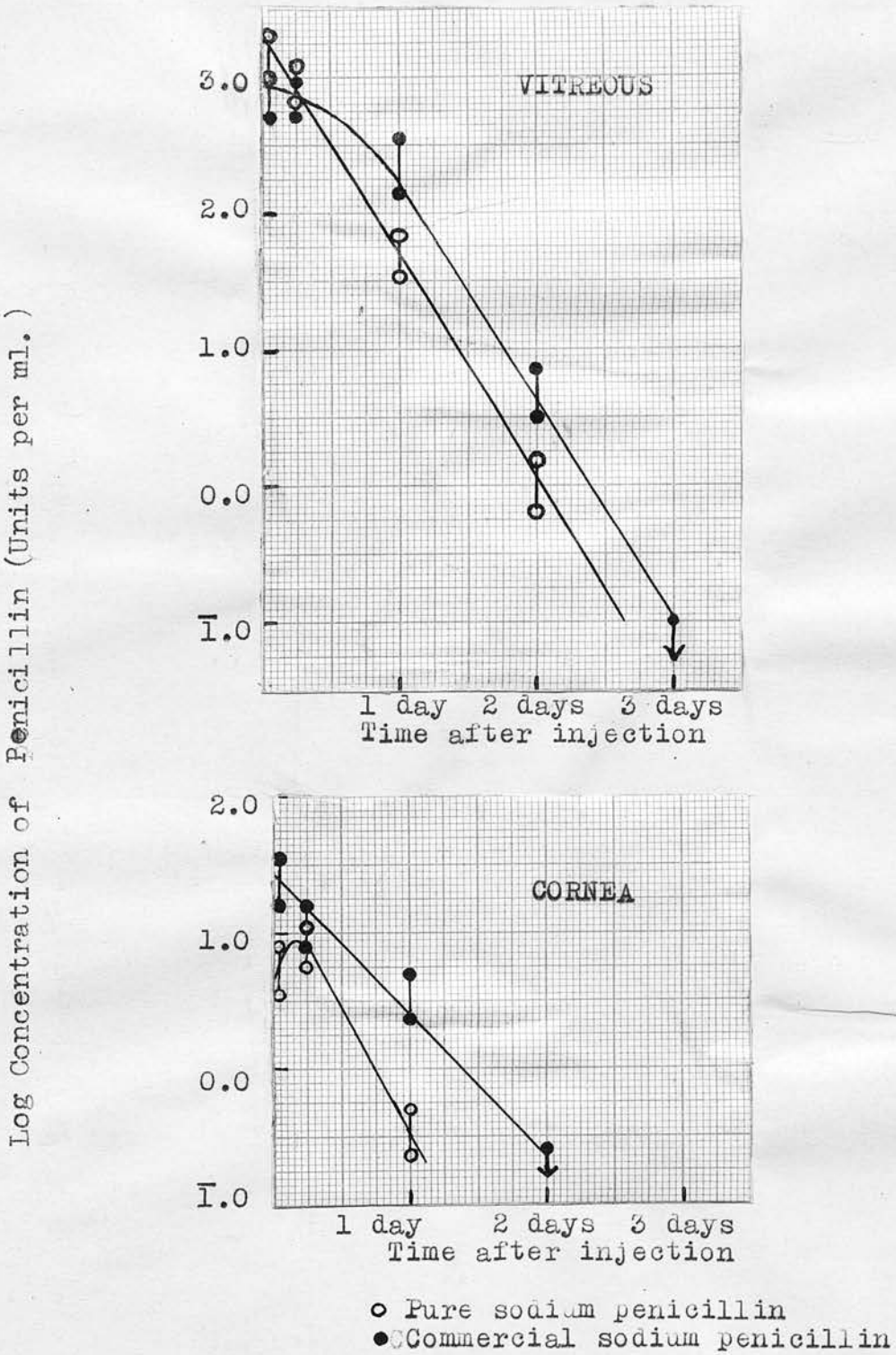
1. Tissues from both eyes analysed together.
2. Conjunctiva from site of injection.
3. Conjunctiva not from site of injection.
4. Contamination makes assay doubtful.

The concentration of penicillin falls from very high levels 1 hour after injection, to concentrations approaching and beyond the limits of detection after three days in the case of the vitreous, and after two days in the case of the aqueous and cornea. Penicillin concentrations of less than $\frac{1}{4}$ u. per ml. in the cornea could not be detected owing to the small bulk of that tissue; where concentrations are less than $\frac{1}{4}$ u. per ml., an indication of the concentration of penicillin in the cornea is given by the level of the drug in the aqueous.

The drug diffuses rapidly from the vitreous into the anterior chamber and cornea, where the maximum levels are reached between one and six hours after injection. The concentration of penicillin in the vitreous is throughout much greater than in the other tissues, and the vitreous acts as a depot replenishing the drug absorbed from the tissues. Penicillin was also detected in the conjunctiva 1 hour and 6 hours after injection, but not after 1 day. Since higher concentrations of penicillin were found in that part of the conjunctiva near the point of injection, it seems likely that most of the drug did not diffuse through the sclera but flowed through the injection puncture.

With commercial sodium penicillin the levels are slightly but significantly higher than with the pure salt. Figure 12a, in which the logarithm of

Figure 12



The clearance of penicillin from the cornea and vitreous, after the intravitreous injection of sodium penicillin.

the concentration in the vitreous is plotted against the time after injection, shows that the difference is due to a delay in the clearance of penicillin from the vitreous during the first day after injection of commercial penicillin. Since the levels in the aqueous and cornea also show the difference, it must be due, not to a decreased rate of diffusion of the commercial salt through the vitreous, but to a slower rate of absorption into the circulation.

In the case of pure penicillin, the log. of the concentration is inversely proportional to the time after injection throughout the whole time range, while with the commercial salt the log. of the concentration is inversely proportional to the time only after the first day, and thereafter the gradient of the line is the same as that for the pure salt. Figure 12b. shows the log. of the concentration of penicillin in the cornea, plotted against the time after injection.

(c) Injection of sodium sulphacetamide and sodium penicillin combined into normal vitreous.

Experiments by Dr. J.P. Duguid showed that incubation of sodium penicillin with sodium sulphacetamide for 24 hrs at 37°C does not destroy any penicillin.

Table 21 shows the results of experiments in which 0.1 ml. of 30 per cent sodium sulphacetamide containing 2,000 units of pure sodium penicillin was injected into the vitreous of normal eyes of rabbits. In two eyes on which assays were made one day after injection, there is no significant difference from the results obtained when the drugs were injected singly. The results for rabbit 1942 two days after injection are considerably less than the results obtained when the drugs were injected singly, while rabbit 1946 under the same conditions gave results similar to those obtained previously.

See over for Table 21.

Time	Rabbit No.	Concentration of Penicillin (units per ml.)			Concentration of Sulphacetamide (mgs/100 gms)				
		Vitreous	Aqueous	Cornea	Vitreous	Aqueous	Cornea	Iris	Sclera
1 day	1941 R.E.	16-32	½-1 [1]	1-2 [1]	145	43.5 [1]	47.8	61.1	17.9
	1941 L.E.	16-32			165		55.3	75.2	107
2 days	1942 R.E.	<⅛	<1/10 [1]	<½ [1]	3.9	2.5 [1]	-	-	8.0
	1942 L.E.	½-1			37.7		0.2	0.9	1.8
	1946 R.E.	6-12 [1]	<1/10 [1]	<½ [1]	55.1	2.9 [1]	2.1 [1]	6.4	
	1946 L.E.				61.4			6.1	-

TABLE 21

[1] Tissue of both eyes analysed together.

Concentration of penicillin and sulphacetamide in the ocular fluids and tissues after the inteavitreous injection of 2,000 units pure sodium penicillin, and 0. 1 ml. 30% sodium sulphacetamide combined, and singly, into the normal eyes of rabbits.

(d) Injection of Sodium Sulphacetamide and Sodium Penicillin into infected Vitreous.

Table 22 shows the results of experiments in which the drugs were injected, both together and singly, into the vitreous of infected eyes of rabbits. Rabbit 1945, 2 days after injection with penicillin and sulphacetamide combined shows concentrations of both drugs without significant difference from the results obtained when the drugs were injected into normal eyes. In rabbit 1951, one eye was infected and one eye was normal, and the tissues taken one day after the injection of penicillin alone, the levels in the vitreous of both eyes is the same. However in rabbits 1943 and 1960, also one day after injection, the drug concentrations are much lower than in normal eyes. Thus in some cases, the drugs disappear from infected eyes after intravitreous injection, faster than from normal eyes.

See Over for table 22

Time	Rabbit No.	Condition	Concentration of Penicillin (units per ml.)			Concentration of sulphacetamide. (mgs/100 gms.)			
			Vitreous	Aqueous	Cornea	Vitreous	Aqueous	Cornea	Iris
1 Day	R.E. 1943	Infected	½ - 1	< 1/5th	<1	9.3	5.7	2.5	1.9
	L.E.	Infected			Perforated.				
	R.E. 1951	Infected	50-100	1/5 - ½	1-2	-	-	-	-
	L.E.	Normal	50-100		Contaminated	-	-	-	-
	1960 R.E.	Infected	-	-	-	56.6	5.4	2.0	8.1
	L.E.	Normal	-	-	-	322	52.3	46.3	62.2
2 Days	R.E. 1945	Infected	2-4[1]	1/10[1]	<¼[1]	112	20.7[1]	19.2[1]	32.2
	L.E.	Infected				80.2			21.0

TABLE 22

1. Tissues from both eyes analysed together.

Concentration of penicillin and sulphacetamide in the ocular fluids and tissues after the injection of 2,000 units pure sodium penicillin and 0.1 ml. 30% Sodium sulphacetamide combined, and singly, into the infected eyes of rabbits.

(e) Subconjuctival injection of Sodium Penicillin and Sodium Sulphacetamide.

The concentrations of the drugs in the ocular tissues and fluids after 30,000 units of commercial sodium penicillin and 0.6 ml. of 30% (W/V) sodium sulphacetamide were injected subconjunctivally into the eyes of rabbits are shown on Table. 23. Very high concentrations of both drugs are found in the cornea and aqueous, one hour after injection, and smaller amounts in the vitreous. After six hours the concentrations of sulphacetamide in the ocular tissues are approaching the limit of therapeutic usefulness, while the concentration of penicillin approaches that limit about 12 hours after injection. There is no significant difference between the concentrations of penicillin found in the tissues of normal eyes and in eyes with experimental corneal ulcers produced by intracorneal injection of Staphlococcus Aureus (Oxford strain)

See Table 23 Over

Time	Rabbit No.	Units /ml. penicillin			Concentration of Sulphacetamide mg /100 gm			
		Aqueous	Vitreous	Cornea	Aqueous	Vitreous	Cornea	Iris
1 hr	1933A Normal	25 - 50	1 - 2	125-250	-	-	-	-
	1955 Normal	-	-	-	240	15.6	437	117
6 hrs	1933A Normal	-	-	-	12.2	1.6	7.2	6.4
	1955 Normal	$\frac{1}{2}$ - 1	1/10-1/5	4 - 8	-	-	-	-
	1962 (Normal	$1\frac{1}{2}$ - 3	1/5 - 2/5	4 - 8	-	-	-	-
	(Infected	$\frac{3}{8}$ - $\frac{3}{4}$	1/5 - 2/5	1 - 2	-	-	-	-
12 hrs	1957 (Normal	$1\frac{1}{10}$ - 1/5	1		-	-	-	-
	(Normal		$\frac{1}{10}$	$\frac{1}{2}$ - 1	-	-	-	-
	1961 (Normal	$< \frac{1}{10}$	$\frac{1}{10}$ - $\frac{1}{5}$	$< \frac{1}{2}$	-	-	-	-
	(Infected	$< \frac{1}{10}$	$\frac{1}{10}$ - $\frac{1}{5}$	$< \frac{1}{2}$	-	-	-	-

TABLE 23 Concentrations of penicillin and sulphacetamide in ocular tissues and fluids after subconjunctival injection of the drug.

(5) The penetration of sodium sulphacetamide into the skin.

(a) Methods of removing hair.

Table 24 shows the results of experiments in the penetration of sodium sulphacetamide in vitro into the skin from sides of rabbits, from which the hair had been removed by different methods. Each figure is the mean of two results.

Method of removing hair	Mgs/100gms. sulphacetamide	
	in the skin	through the skin
Plucking	293	1.7
Scissor trimming	300	1.4
Depilating paste	577	3.0

TABLE 24

The effect of various methods of removing hair on the absorption of 10% sodium sulphacetamide in 15 minutes by isolated rabbit's skin in vitro. Each figure is the mean of 2 experiments.

There is no significant difference between the results for plucked and scissor trimmed skin, but the depilating paste, which also caused oedema of the skin and fascia, increased the penetration of the drug. Plucking was therefore chosen as a safe and convenient method of removing hair in subsequent experiments. Only certain rabbits are

suitable for plucking, in others where the hair offered resistance to plucking the skin was broken and such rabbits were not used.

(b) Comparison of species.

The same technique was used to compare the penetration of sodium sulphacetamide into the skin of various species, and the results for these experiments are shown on Table 25. Rabbit skin is the most permeable to the drug with the permeability decreasing in the order, guinea pig, human, rat;

Species	Mgs/100gms. sulphacetamide.	
	in the skin	through the skin
Rabbit	340 ± 45	1.5 ± 0.4
Guinea Pig	220 ± 19	0.8 ± 0.3
Rat	94 ± 21	0.7 ± 0.2
Human	165 ± 45	1.0 ± 0

TABLE 25

The absorption of 10% sodium sulphacetamide in 15 minutes by the isolated skins of different species in vitro. Each figure is the mean of four Experiments.

± Standard deviation.

guinea pig skin is that which is most similar to human skin, as far as permeability to sodium sulphacetamide in concerned,

(c) The effect of wetting agents on the penetration of sodium sulphacetamide into normal, saline treated, and infected skin.

Table 26 shows the concentrations of sulphacetamide in the skin and fascia of guinea pigs, after the application of 10% (W/V) sodium sulphacetamide with and without 0.1% dodecyl sodium sulphate for 15 minutes and 24 hours in vivo to normal skin, scarified skin, infected skin, and to skin which had been treated with saline for 24 hours. The results show that application of the drug with the wetting agent for 15 minutes and one day to normal skin does not affect the penetration significantly (guinea pigs, 8, 9, 39, 46, and 49), but with saline treated and infected skin, there is a marked increase in the drug concentration in the tissues, particularly in the skin, when the drug was applied for 24 hours with the wetting agent (guinea pigs 42, 43, 37, 38 and 39). The experiments on guinea pigs 27 and 28 in which the drug was applied for 15 minutes to normal and saline treated skin on the same animal, indicate that after the application of saline for 24 hours, the penetration of sodium sulphacetamide into the skin is increased. The concentration of sulphacetamide found in infected skin and fascia after 15 minute application of the drug is in the the same region as that found after 24 hours application of the drug. This is probably due to a rapid uptake of the drug by the wound and absorption into the circulation, and to the partial healing

Time of Application	Guinea Pig Number	Concentration of Sulphacetamide (mg/100gm.)															
		Normal				Scarified				Saline Treated				Infected			
		+W.E.		- W.E.		+W.E.		- W.E.		+W.E.		- W.E.		+W.E.		- W.E.	
		Skin	Fascia	Skin	Fascia	Skin	Fascia	Skin	Fascia	Skin	Fascia	Skin	Fascia	Skin	Fascia	Skin	Fasc
	8	29.1	2.2	21.8	2.5	-	-	-	-	-	-	-	-	-	-	-	-
	9	18.3	5.8	20.5	4.2	-	-	-	-	-	-	-	-	-	-	-	-
	11	-	-	14.7	2.7	740	43.5	754	30.0	-	-	-	-	-	-	-	-
15 mins.	12	-	-	17.8	2.3	735	80.5	628	135	-	-	-	-	-	-	-	-
	18	-	-	12.0	2.0	341	30.4	500	46.6	-	-	-	-	-	-	-	-
	27	-	-	13.0	0.6	-	-	-	-	-	-	40.1	9.4	-	-	-	-
	28	-	-	9.0	1.6	-	-	-	-	-	-	20.5	1.8	-	-	-	-
	29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	163	16.
	31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	123	11.
	46	47.6	7.6	47.1	7.4	-	-	-	-	-	-	-	-	-	-	-	-
	49	161	14.8	94	12.3	-	-	-	-	-	-	-	-	-	-	-	-
	39	128	6.0	97	4.5	-	-	-	-	-	-	-	-	-	-	-	-
24 hours	42	-	-	-	-	-	-	-	-	213	8.7	90.3	5.9	-	-	-	-
	43	-	-	-	-	-	-	-	-	277	36.2	134	22.8	-	-	-	-
	37	-	-	-	-	-	-	-	-	-	-	-	-	284	14.2	120	8.
	38	-	-	-	-	-	-	-	-	-	-	-	-	404	29.1	191	11.
	39	-	-	-	-	-	-	-	-	-	-	-	-	301	14.5	117	10.

+W.E. 10 per cent (W/V) sodium sulphacetamide 0.1 per cent (W/V) dodecyl sodium sulphat

- W.E. 10 per cent (W/V) sodium sulphacetamide.

TABLE 26. The effect of a wetting agent on the penetration of sulphacetamide into skin, from 10 per cent (W/V) sodium sulphacetamide applied for fifteen minutes and twenty four hours, to normal, scarified, saline-treated, and infected guinea pig skin, in vivo.

of the wound after 24 hours.

Tables 27a and b show the results for two experiments in which 10% (W/V) sodium sulphacetamide was applied for 24 hours to the normal skin of a rabbit *in vivo*.

Time after treatment started.	Concentration of Sulphacetamide (gms. percent)		
	Rabbit I	Rabbit II	Mean
0 hours	9.2	9.2	9.2
1 hour	9.4	9.1	9.25
3 hours	9.4	9.2	9.3
5 hours	8.0	7.4	7.7
24 hours	4.4	3.7	4.05

TABLE 27a

The uptake of 10% sodium sulphacetamide by rabbit's skin *in vivo* during twenty-four hours application.

Note:- 9.2 gms. per cent sulphacetamide is equivalent to 10 per cent sodium sulphacetamide.

See over for Table 27b.

Tissue etc.	Concentration of Sulphacetamide (mgs.percent)		
	1676A Rabbit	1677A Rabbit	Mean
^{1}Skin	54.1	70.1	62.1
^{1}Fascia	14.8	26.4	20.6
^{1}Muscle	4.9	1.5	3.2
^{2}Skin	-	26.0	26.0
^{2}Fascia	-	15.1	15.1
Blood (Total)	1.2	0.94	1.07

TABLE 27b.

Concentration of sulphacetamide in the blood, skin and subcutaneous tissues after the application of 10 per cent sodium sulphacetamide to rabbit's skin for twenty-four hours.

Note:- ^{1}Skin came from the part to which the drug was applied and ^{1}fascia and ^{1}muscle were lying directly under it; ^{2}skin was immediately adjacent to ^{1}skin and ^{2}fascia was lying directly under it.

The concentrations of the drug in the skin and fascia underlying the applied solution are of the same order as is found in similar experiments on guinea pigs (see Table 26). Small but definite amounts of the drug are found in the blood and

underlying muscle, while fairly high concentrations are found in the skin and fascia immediately adjacent to the area to which the drug was applied. In both rabbits, the concentrations of the drug in the applied solutions do not change perceptibly until five hours after application, and after that, the drug concentrations fall in 24 hours to approximately half the original value.

The results for similar experiments with the application to guinea pigs' skin, of sodium sulphacetamide alone, and in the presence of dodecyl sodium sulphate, for three days, are shown on Tables, 28a, b, and c. Columns marked * indicate that the skin and superficial fascia were oedematous /

oedematous and inflamed after 72 hours, and such damage is found in skins treated with and without the wetting agent. No marked trend in the disappearance of the drug, as in the case of the experiments with rabbits, can be inferred from these results; nor is there any significant difference in the disappearance of the drug when it was incorporated with the wetting agent. In most cases, the concentration of the drug in the applied solution increased between 48 and 54 hours after the application commenced, probably due to evaporation, or to the absorption of water from the drug solution; this effect is greater in those cases in which the skin and fascia were oedematous. The actual diminution in volumes of the drug solutions are also given but these figures are not reliable since it was impossible to avoid losses.

See Over for Table 28A

Time	Concentration of Sulphacetamide gms/100gms.					
	Guinea Pig 55		Guinea Pig 56		Guinea Pig 57	
	+	-	+	-	+	-
0 hours	9.1	9.1	9.1	9.1	9.1	9.0
6 hours	8.2	7.0	8.0	7.1	-	-
24 hours	-	-	-	-	7.8	9.0
30 hours	7.5	7.5	6.2	7.6	7.3	-
48 hours	7.5	6.9	6.1	7.9	6.9	11.2
54 hours	13.0	7.0	6.7	8.5	5.8	9.0
72 hours	9.1	8.1	6.8	9.3	5.2	9.2
Diminution in volume.	1.75ml	1.75ml.	1.25ml.	1.5ml	2.3ml.	1.7ml.

\+ 10 per cent sodium sulphacetamide with 0. 1 per cent dodecyl sodium sulphate.

\- 10 per cent sodium sulphacetamide alone.

TABLE 28A

The disappearance of sulphacetamide from a solution of 10 per cent sodium sulphacetamide applied to the skins of guinea pigs for 72 hours.

Table 28b shows the concentration of the drug in the tissues underlying the drug solutions and immediately adjacent to it. Small, but significant, concentrations of the drug were found in the adjacent tissue, while in the tissue underlying the drug, in two cases the concentration of the drug in the skin was increased in the presence of the wetting agent and in the other case there was no significant difference.

Tissue		Concentration of Sulphacetamide (mgs. per cent)					
		G.-pig 55		G.-pig 56		G.-pig 57	
		*+ W.E.	- W.E.	+ W.E.	*- W.E.	+ W.E.	- W.E.
Subjacent	Skin	250	123	241	101	134	128
"	Fascia	9.0	5.1	5.6	4.2	8.4	9.8
Adjacent	Skin	4.1	3.8	4.8	17.4	14.7	5.7
"	Fascia	5.5	2.4	2.4	4.4	5.1	2.5

Columns marked * indicate that the skin and fascia were inflamed and oedematous at the end of the experiment.

TABLE 28b

The effect of 0.1 per cent dodecyl sulphate on the concentrations of sulphacetamide in the skin and superficial tissues after the application of 10 per cent sodium sulphacetamide for three days.

Twenty-four hour urines were collected from guinea pig 57 and the total sulphonamide concentrations in these urines and the blood total sulphonamide concentration at 72 hours are shown in Table 28c.

Time		Voume (ml)	Total Sulphacetamide (mgs.)	Conc. of total Sulphacetamide (mgs. per cent)
Urine	24 hrs.	5.1	1.76	35
	48 hrs.	5.2	2.51	49
	72 hrs.	20.0	3.22	16
Blood	72 hrs.	-	-	1.6

TABLE 28c

Concentrations and total amounts of Total Sulphacetamide in blood and urine after and during the application of 10 per cent sodium sulphacetamide to the skin of guinea pig 57 for 72 hours.

(d) The rate of removal of sulphacetamide from the Skin.

Table 29 shows the concentrations of sulphacetamide retained by normal and grazed guinea pig skin, one day after the local application of sodium sulphacetamide. In normal skin, the concentration of the drug is approximately halved in 24 hrs. Although higher concentrations are found in damaged skin, the rate of removal is much greater in normal skin.

Guinea Pig Number	Time after application	Concentration of Sulphacetamide (mg/100gm)			
		Normal Skin		Damaged Skin	
		Skin	Fascia	Skin	Fascia
22	0	11.8	5.7	1190	92
	24hrs	6.6	1.0	15.8	3.9
23	0	19.0	4.2	760	171
	24hrs	8.1	0.7	25.1	4.1

Table 29 Concentrations of sulphacetamide in the skin and fascia, twenty-fours after and immediately after the application of 10 per cent sodium sulphacetamide for fifteen minutes in vivo, to normal and severely grazed guinea pig skin.

D I S C U S S I O N.

The main part of the work has been concerned with the effect of wetting agents on the penetration of drugs into the cornea and the skin, and the distribution of drugs in the ocular tissues after intravitreous injection.

In assessing the value of topical application of drugs to the cornea in humans, from experiments on rabbits, it must be remembered that the rabbit cornea is thinner than the human cornea, and therefore drugs penetrate through it more rapidly (Friede,1934).

The use of wetting agents to increase the penetration of drugs through the cornea was first described by O'Brien and Swan (1942); Leopold and Scheie (1943) and Bellows and Gutman (1943) were first to report the increased penetration of sulphonamides through the cornea, from ointments, pastes, and microcrystalline suspensions containing detergents.

With sodium sulphacetamide applied in drops to the eye, the beneficial effects of incorporating a wetting agent in the solution would be greatest in infections involving the cornea and iris. In these tissues the period during which effective levels of the drug are maintained after a single

application of drops is extended from fifteen minutes or less, to more than two hours. Robson and Scott (1944) have reported that dodecyl sodium sulphate increased the effectiveness of 10 per cent (W/V) sodium sulphacetamide and 10 per cent (W/V) sodium sulphadiazine in the treatment of experimental corneal infections in rabbits. In their experiments, the drugs were applied in drops at hourly intervals. Since the time between infection and the establishment of bacteriostatic concentrations of the drug in the diseased tissues is an all-important factor, it is probable that the beneficial effect observed with the wetting agent was due, to a large extent, to the attainment of effective concentrations of the drug after one application, compared with two, without the wetting agent. Another factor would be the deeper penetration of the drug when applied with the wetting agent. Only a very small proportion of the drug applied is found in the ocular tissues, the greater part of the drug flowing away through the lachrimal duct; this proportion is increased slightly when the drug solution contains a wetting agent. It is possible that an improvement similar to that with a detergent could be obtained by making the second application of the drug, without the wetting agent, fifteen minutes after the first. In the case of a drug such as V.187, which does not by itself penetrate

the cornea to the same extent as sodium sulphacetamide, application with a wetting agent would be particularly valuable. The use of Lissapol N offers the advantage that it forms permanent solutions in the presence of high concentrations of the drugs, and it does not lose its effectiveness in increasing the penetration of drugs through the cornea, on standing. Aerosol OT, probably due to temperature differences manifests its action only in vivo, and not in vitro.

It has been shown (Trevan and Boock, 1927) that local anaesthetics penetrate into the cornea only as the free base, and not in ionic form. In 1929, Fischer showed that the corneal epithelium is a barrier to the passage of drugs into the cornea. More recently, Swan and White (1943) have shown that the epithelium acts as a membrane with differential permeability, presenting a greater barrier to some substances than to others. The wetting agents act by inducing reversible changes in the corneal epithelium, which is rendered more permeable to the drugs. This finding has been confirmed by Leopold (1945 b). Repeated or prolonged application of high concentrations of detergents results in mucoid discharge from the cornea and even removal of the corneal epithelium. Bile salts have been used experimentally to remove the corneal epith-

elium (Friedenwald et al, 1944), and cyclamin, a saponin derivative, has been used to remove the epithelium from tadpoles (Martensson, 1936). There is evidence that the local application of sulphonamides to the eye deters the regeneration of the corneal epithelium (Berens et al, 1943; Bellows and Gluckman, 1943; Leopold and Steele, 1945); thus prolonged or repeated application of wetting agents with sulphonamides to the eye is contraindicated, and although they do increase the penetration of drugs applied from a corneal bath, they should not be used in that way.

The increase in the permeability of the cornea caused by contamination with vesicants used in chemical warfare, is probably due to damage to the corneal epithelium; that N-methyl-di (2 chloroethyl) amine takes two to three days to show the increase, compared with less than one day with dichloroethyl sulphide, may arise from the fact that while N-methyl-di (2 chloroethyl) amine was applied as an aqueous solution, dichloroethyl sulphide was applied in liquid paraffin. Without further work to confirm this interpretation, the possibility of a real difference between the action of the vesicants cannot be ignored.

High levels of drugs can be reached in the ocular tissues by sub-conjunctival injection. The amount of drug reaching the anterior chamber

after subconjunctival administration of micro-crystalline sulphathiazole can be increased incorporating a capillary dilator, such as histamine, in the suspension (Leopold and Scheie, 1943).

In one experiment in which 0.6 ml. of 30 per cent (W/V) sodium sulphacetamide was injected subconjunctively into a rabbit, the concentration of the drug found in the anterior chamber six hours later was 12.2 mg. per 100 gm, which is adequately bacteriostatic. The bleb from the injection had not cleared in six hours, but no toxic reaction was apparent.

With subconjunctival injection of penicillin, Struble and Bellows,(1944) found that there was practically no penicillin left in the ocular tissues, three hours after the administration of 2,500 units. Using the same dose of penicillin, von Sallman (1945) found effective levels only in the aqueous, four hours after injection, which compared unfavourable with the concentrations reached after iontophoresis or cotton pack application. Leopold (1945a) found higher concentrations in the vitreous following subconjunctival injection of penicillin, in cases where the anterior chamber was experimentally infected. Rycroft (1945b) found penicillin in the vitreous after the injection of 4,000 units of penicillin subconjunctivally in infected human eyes.

In the experiments carried out in the present work, much greater amounts of the drug were used - 30,000 units of commercial penicillin were injected subconjunctively. Effective concentrations of penicillin were found in the aqueous, cornea, and vitreous, twelve hours after injection. In experiments on eyes with experimental corneal ulcers, the results were not different from those for normal eyes. The subconjunctival injection of such large doses of commercial sodium penicillin causes a yellowish subconjunctival bleb which is absorbed leaving no trace and no marked reaction after twelve hours.

It has been found that drugs injected into the vitreous are slowly absorbed and remain in the ocular tissues for considerable periods. Von Sallman et al (1944) have found that antibacterial activity persists in the vitreous for at least twenty four hours after the direct intravitreous injection of 500 units of penicillin. Mann (1946) found 0.7 units of penicillin per ml. in the vitreous, and 0.06 units per ml. in the aqueous, two days after the injection of 1,000 units of penicillin into the vitreous of a rabbit.

These findings have been confirmed. Antibacterial concentrations of penicillin and sulphacetamide persist for two days in the cornea and aqueous,

and for three days in the vitreous, after the injection of 0.1 ml. of 30 per cent (W/V) sodium sulphacetamide or 20,000 units per ml. sodium penicillin, either together or singly into the vitreous of a rabbit. Infection of the vitreous, in some cases, increases the rate of clearance of the drugs.

The results for sulphacetamide differ from those for penicillin in two respects: 1) The rate of clearance of sulphacetamide is slightly slower than that of penicillin. 2) The ratio of the concentration of penicillin in the vitreous to that in the aqueous and the cornea (when both pure and commercial penicillin was injected) was approximately 100:1 at one hour, six hours and one day, and of the order of 10:1 at two days; the ration of the concentration of sulphacetamide in the vitreous to that in the aqueous and cornea is approximately 5:1 from 1 hour to three days.

If the assumption is made that there is no selective barrier to the passage of pencillin from the vitreous to the anterior chamber, which does not hinder the passage of sulphacetamide (which seems unlikely in view of 1) above), or that penicillin is not destroyed in the anterior chamber, these differences may be explained by a) a rate of diffusion of penicillin through the vitreous slightly greater than

than that of sulphacetamide, and b) a rate of removal of penicillin from the anterior chamber, much greater than that of sulphacetamide.

Von Sallman et al (1944) found that while the intravitreous injection of pure penicillin caused only slight and transitory reaction, crude penicillin was more irritating and produced damage to the retina. Rycroft (1945a) found that 2,000 units and 5,000 units of penicillin are well tolerated by infected human vitreous, and Leopold (1945a) has found that 2,500 units are tolerated by infected rabbit vitreous. On the other hand, Mann (1946) has reported that merely breaking into the vitreous gel may cause permanent opacity in a rabbit.

The delay in the absorption of commercial penicillin compared with pure penicillin may be associated with the toxic effects of the impure product; this observation is especially interesting in view of the present lack of knowledge of the blood-aqueous barrier with respect to penicillin (Sorsby, 1945).

There is no doubt that high concentrations of drugs are maintained for longer in the ocular tissues after intravitreous injection, than with any other method of administration. Until the extent of its toxic effects are definitely established, the intravitreous injection of penicillin or sodium sulphacetamide can be used only in the treatment of

deeply infected eyes which could not be saved by other methods of administering the drugs.

Since the action of sulphonamides is bacteriostatic, for effective action they must be maintained at the site of infection for considerable periods. The work of Biggar (1944) and Garrod (1945) indicates that the action of penicillin is bacteriocidal rather than bacteriostatic. The latter author concludes that there is nothing to be gained by using high concentrations of penicillin in local treatment, except where it means that loss by escape, dilution, or absorption shall not permit the concentration to fall below the minimum level for full effect. This condition requiring the use of strong penicillin solutions is found in the treatment of ocular infections. If the initial level of the drug exceeds the effective concentration, the presence of the minimum effective concentration will be prolonged, so that deeper foci of infection are likely to be benificially influenced. Since there is some evidence to show that high concentrations of penicillin are more effective than low concentrations in dealing large numbers of organisms (Hobby and Dawson, 1944; Rantz and Kirby, 1944), and that certain organisms are inhibited only by relatively high concentrations of penicillin (Stewart,1945), the use of strong penicillin solutions would be particularly useful under these conditions.

It has been known for a long time that the skins of fishes and amphibians are freely permeable to water and water soluble substances (Rothman, 1929). Lovatt Evans (1945) ~~Starling (1926)~~ stated that " it may be regarded as established that the uninjured skin is impermeable for watery solutions of salts or other substances". This statement is not universally true for it has been shown that lipoid soluble substances such as salicylic acid are capable of diffusing through unbroken skin when water is the solvent (Leslie-Roberts, 1928); it is probable that only the lipoid-soluble free base is taken up by the normal skin.

The results presented show that sulphacetamide is rapidly taken up by the skin during the topical application of sodium sulphacetamide. Rabbit skin is more permeable to the drug than guinea pig, human, and rat skin, in decreasing order of permeability; this is in good agreement with the results of Strakosch et al (1943).

The permeability of the skin is greatly increased when the stratum corneum is abraded or the skin is broken. Even with undamaged skin, definite amounts of the drug are found in the superficial fascia after the application of 10 per cent (W/V) sodium sulphacetamide for 15 minutes to guinea pig skin. Slightly greater amounts of the drug are taken up by skin which has been treated for 24 hours with saline. It is possible that the prolonged application of the saline causes the keratin of the

stratum corneum to swell and thus increases its permeability. There is also evidence that after the application of aqueous solutions to the skin of a guinea pig for as long as two days, there is slight absorption of water by the skin.

The drug is slowly taken from normal skin into the circulation. The concentration of sulphacetamide in normal skin, twenty-four hours after application, is approximately half the concentration found immediately after the applied solution was withdrawn. With damaged skin, the rate of removal is much greater although high concentrations are still found in the skin and fascia, twenty-four hours after the drug was applied. These results are in good agreement with those of Strakosch and Clark (1943b)

Administration of the drug to the skin with a wetting agent results in an increase in penetration, after application for at least two days, or after one day to skin which had been treated for the previous 24 hours with saline. Other workers have reported the action of wetting agents increasing the penetration of drugs through the skin. Pereyra (1945) found that wetting agents increased the penetration of copper from solutions of copper sulphate applied to penile skin. Mc.Kee (1943,a,b.) et al have described bases, which they call penetrasols, for the

application of sulphonamides to the skin. Wetting agents are important constituents of these bases from which the drugs penetrate into the cutis in amounts detectable by histochemical methods. Using other bases, the greatest part of the drug is concentrated in the corneum stratum.

Wetting agents and detergents cause effects in many biological systems. For example, they inactivate viruses (Bawden and Pirie, 1938), denature proteins (Anson, 1939), potentiate the bacteriocidal action of phenols (Ordal et al,1941 have lytic (Bayliss, 1937) and bacteriocidal actions (Domagk, 1935a).

In experiments on insecticides, Hurst (1940,1944) has shown that the permeability of the insect cuticle, and particularly the epicuticle, is increased when the medium in which the insecticide is dissolved is a fat solvent. This he attributes to van der Waals' interaction between the fat solvent molecules and the lipoids of the epicuticle, resulting in the production of a relatively open three-dimensional system, in which the solvent molecules participate, functionally. This conception applied to the action of wetting agents in increasing the penetration of drugs through the cornea and the skin is supported by the work of Schulman and co-workers (1935,1937,1939), who have found that wetting agents injected under a lipoid monolayer increase the surface pressure of the

film. This is interpreted as the formation of a mixed film of the detergent and the lipoid, firstly by interaction of the polar heads, leading to van der Waals' interaction between the hydrophobic elements. These results have been correlated with the lytic action of the wetting agents. Baker et al (1941) have suggested that the antibacterial action of detergents is preceded by a disorganisation of the cell membrane due to their surface activity. If such is the mode of action of the wetting agents on corneal epithelium the penetration of the drug into the cell is increased. An alternative mode of action might be the suspension of the intracellular cement by the wetting agent. Trim and Alexander (1944) have produced evidence showing that the action of wetting agents in increasing the penetration of hexyl resorcinol into ascaris is associated with compound formation between the wetting agent and the drug. No evidence was found to indicate that such is the case in the penetration of sulphonamides through skin and cornea.

S U M M A R Y

(1) Various methods of applying drugs locally to the eye and skin *in situ*, and to the isolated tissues *in vitro* are described.

(2) Methods are described for the production of experimental infections in the cornea, vitreous, and skin.

(3) Simple methods are described for the measurement of diffusion constants (in gels), and for the comparison of interfacial tensions.

(4) Wetting agents increase the penetration of topically applied drugs into the ocular tissues. Of the wetting agents studied, dodecyl sodium sulphate is the most effective; lissapol N is also effective, and offers the advantage that (unlike dodecyl sodium sulphate) it forms stable solutions in the presence of high concentrations of drugs.

(5) The use of dodecyl sodium sulphate with sodium sulphacetamide would be beneficial in infections involving the cornea and iris.

(6) Wetting agents have no effect on the diffusion of sodium sulphacetamide through gels, and no relationship is found between the interfacial tension of drug-detergent solutions at air/water and paraffin/water interfaces, and the increased penetration of the drug into the eye.

(7) Wetting agents cause damage to the corneal

epithelium, which permits greater penetration of topically applied drugs into the ocular tissues.

(8) The contamination of eyes with dichloroethyl sulphide and N-methyl di(2 chloroethyl) amine increases the permeability of the cornea to sodium sulphacetamide. With the former vesicant, the increase takes place less than one day after contamination, with the latter, it takes place about three days after contamination.

(9) After intravitreous injection of sodium sulphacetamide and/or sodium penicillin, therapeutic levels are maintained in the vitreous for three days, and in the aqueous and cornea for two days. Where the vitreous is infected, in some cases, the rate of clearance of the drugs from the eye is greater.

(10) Therapeutic levels of penicillin are maintained in the aqueous, cornea, and vitreous, for twelve hours after sub-conjunctival injection of 30,000 units of sodium penicillin.

(11) Sulphacetamide penetrates into intact skin from a solution of sodium sulphacetamide, applied locally for fifteen minutes. Where the skin is broken, by grazing or scarification, the penetration of the drug into and through the skin is much greater.

(12) Twenty four hours after the application

of the drug to intact skin, the concentration of sulphacetamide in the skin is halved.

The absorption of the drug into the circulation during the first day after application is much greater when skin is broken.

(13) Wetting agents increase the penetration of sulphacetamide into the skin when applied for two days to normal skin, or for one day to skin which has been treated for twenty-four hours with saline.

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